Downstream Processing
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Lecture - 38
Stabilisation, Utilities and Other Auxiliary Processes and Absorption

Once you have made bioproduct; that means a biopharmaceutical or a biological molecule, you would like to keep it for certain amount of time until it is used by the customer; that means, it should have some amount of shelf life. The shelf life may vary from days to even months depending upon when the customer would like to make use of the product.

So, all these products should have certain amount shelf life; that means, it should not lose it is activity, it should not get degraded or it should not react with the other impurities found in the product or it should not have bacterial growth or fungal growth, which may spoil the product itself, So, we need to stabilize the product, and that is the final step in the downstream processing. So, the stabilization could be a physical process, it could be a chemical process or it could be a biological process. There are different approaches by which we could stabilize the molecule away of your interest.

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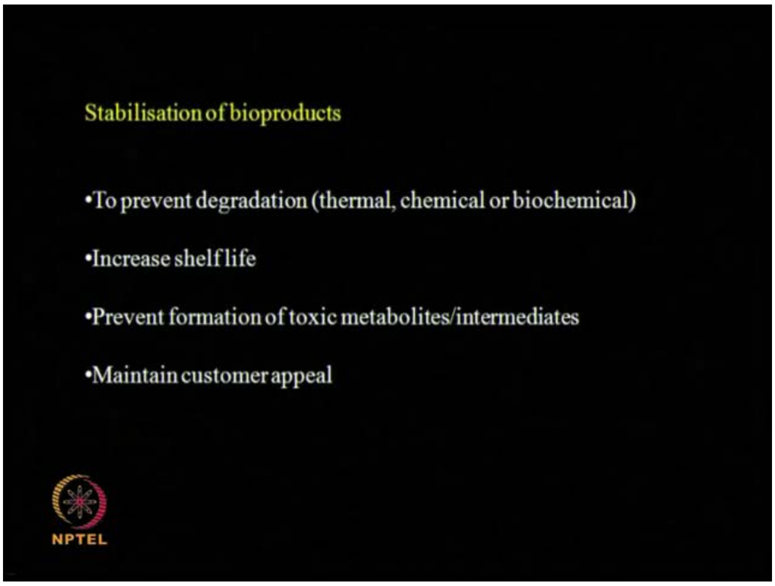

So, the idea of stabilization is to prevent degradation, it could be a thermal degradation, it could be a chemical degradation or a biochemical degradation or it you would like to increase shelf life, as I said you would like to have the product under active conditions as well as in an acceptable form for quite a long period of time. So, you would like to increase the shelf life for example, you might have made a solid product, but if it is slowly becomes a liquid for a period of time the customer might not be interested in taking it as, their final raw material.

They would rather have a solid rather than a liquid. You would like to prevent formation of a toxic metabolites or even intermediates, if the product slowly starts degrading and it form certain intermediates which may be toxic, then that might not be desired. For example, it may happen in pharmaceutical product, your product may be non-toxic harmless, it could be acceptable, it could be a biocompatible and so on. But over a period of time as it starts degrading, the metabolites may be toxic metabolites may not be biocompatible.

So, there could be a issue in that actually, then you want to maintain the customer appeal you would like to have a beautiful looking product, if it is a solid it should be free flowing of acceptable color, texture, uniform, particle size and so on. So, the customer appeal is very, very important and you would like to maintain the customer appeal, until the product is kept in the shelf, which may run into days or even months. So, because of all these reasons you would like to stabilize the bioproduct.

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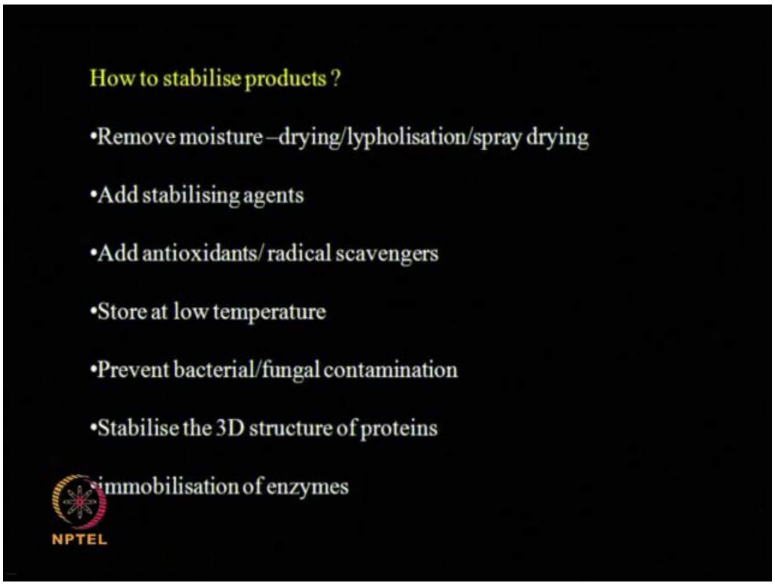

So, there are different ways of stabilizing and we will look at some of these approaches. How do you stabilize these products, one of them is to remove the moisture, moisture could be really problematic to the product because the moisture may help in the growth of bacteria or fungus. The moisture if it has dissolved oxygen may lead to oxidation of your product, if there is any other impurities present, moisture will allow the impurities to dissolve and then react with your product.

So, removal of moisture is very, very important, so how do you do that, we have looked at a different ways of removing moisture we looked at approach call drying, where you heat it up. So, that all the moisture is removed or we talked about lypholisation, where you bring it down to very low temperature. So, that the water becomes solidize which is then removed by vaporization, almost like a sublimation. Then we looked at spray drying where by spraying it and passing hot air you are removing all the moisture.

So, removal of moisture for example, your coffee, instant coffee beautiful looking solids, free flowing solids is done through a spray drying and because the moisture is completely removed. And if you remove the moisture completely and pack it up in inner atmosphere a coffee powder, remains as a powder even for a very long period of time if it is not opened or if the seal is not broken it will always remain whereas, a customer would not like to have agglomerates or solid precipitates of this coffee powder.

Not only the customer appeal, but also the solubility of this particular product, becomes poor if it has formed agglomerate. Second approach to stabilize your product you can add stabilizing agent, there could be agents which may prevent, certain reactions like a scavengers. So, we can add stabilizing agents. Third approach is to add antioxidants; that means, agents which prevent oxidation; that means, they scavenge the oxygen, there by they prevent the oxidation of your product.

You could add radical scavengers, radical is formed because of the u v light. So, if a product is expose to U V light or even sunlight, radicals are formed. So, these radicals could lead to further propagation, leading to more radical formation and more degradation. So, you could add radical scavengers, which will just scavenge the radicals or absorb the radicals and prevent the reaction progressing further, this type of radical initiated reaction progressing further.

Storing at low temperature, may be can maintain products at below room temperature or even 0 degrees temperature, even very low temperature minus 20 degrees, if they are enzyme type of a products. So, by storing it at lower temperature you are preventing the degradation of the product; that means, the activation energy, is controlled by storing it at low temperature. We can add antibacterial, antifungal agents, so that the bacterial growth or fungal growth could be prevented.

We can add agents which will maintain the three dimensional structure of the protein, by doing that you are maintaining the activity of the protein because once the protein loses this three dimensional structure, it is going to lose activity, protein may be present as agglomerate, but the protein activity will be very, very poor. Immobilization of enzymes, as you know enzymes in their native form or when it is in the buffer will slowly lose activity because the three dimensional structure of the enzyme gets disturbed, whereas if you immobilize this enzyme, you are able to maintain the three dimensional structure.

So, the activity of the enzyme, remains over a very long period of time. When you immobilize the activity of the enzyme may be much less than the native enzyme, but activity remains much, much longer. So, there are different ways of immobilizing an enzyme and we are going to look at some of the ways after sometime.

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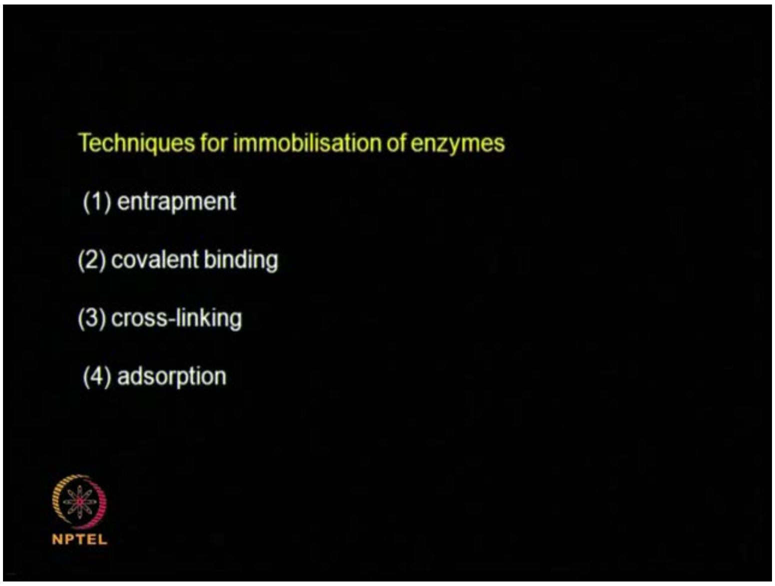

So, how do you immobilize enzymes, as I said there are different ways most important are fore the entrapment; that means, you can entrap the enzyme inside a matrix. So, that it is not being exposed to harsh environment may be p H may be some toxic chemicals may be solvents and so on. Actually, that is called entrapment another one is covalent binding. So, what you do you bind it to some material through a covalent linkage.

So, the enzyme does not lose it is three dimensional structure to match; that means, you are restricting it is flexibility or movement. So, that it does not collapse, the structure does not collapse and there is cross linking you can cross-link enzymes with other, enzymes, other proteins again you are preventing it is collapse of the three dimensional structure. Fourth approach is adsorption, adsorption on different material, so here you are using non bonded interaction; that means, it is not forming a covalent linkage, but it just getting adsorb.

So, the advantage here vis-a-vis the covalent linkages, the activity on the enzyme will be much higher whereas, when you are doing a reaction and forming a covalent bond may be losing the activity of the enzyme, but one disadvantage with adsorption is the enzyme may slowly get leased out over a period of time because it is not covalently bond; that means, it is not strongly bond to the support.

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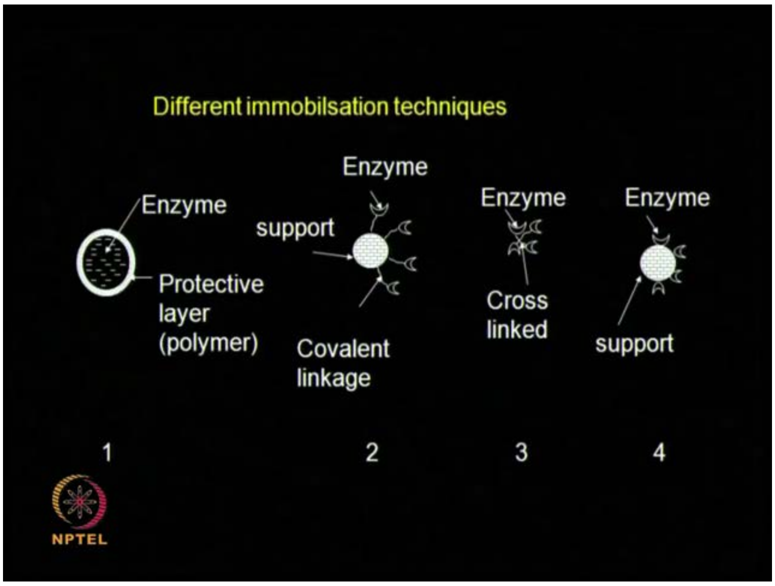

So, these 4 techniques I mention in the previous slides are shown pictorially, in this particular slide. So, as I said entrapment there is a protective layer, like your enzyme may be inside and there is a protective layer surrounding it. So, the enzyme is a protected from the harsh solvents or harsh p H and so on actually. So, one disadvantage here is your reactant has to diffuse in and then react with the enzyme.

So, the rate of reaction is also controlled by the diffusion of the reactants here, then the covalent linkage you are connecting the support with the enzyme, through a your permanent covalent bond. So, you can first react the support, create active size and then the enzyme is attach to this active size. So, that is called the a covalent linkage of enzyme. Third one is cross linking; that means, you can connect one enzyme with another through bonds through a cross linked bonds.

So, you can have cross-linked enzymes and the stability of the enzyme or the three dimensional structure of this enzyme are maintained because of this type of cross linking. Fourth approach is your adsorbing enzyme on to a solid support. So, you have you do not have here, a permanent covalent bond, but you have only adsorption forces. So, these are the different ways of immobilizing enzyme, as I said each one of them have advantages disadvantages over the other you select the technique based on the nature on the enzyme, how long you would like to have the enzyme activity maintained and so on; actually and the cost as well of course.

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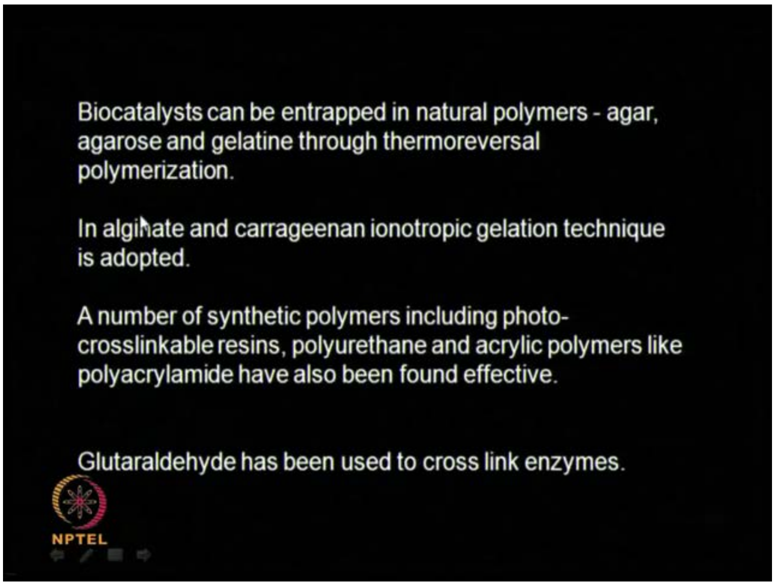

So, for example, if you look at biocatalyst, they can be entrapped in natural polymers natural polymers as you know, are very compatible. So, they do not or they will not affect the activity of the enzyme like agar, agarose, geletine all these can be used by a thermo reversal polymerization technique. Another, natural polymer is alginate, carrageen an. So, all these can be used and make the enzyme entrapped using ion tropic gelatin technique.

You can also use synthetic polymers, polymers which can be polymerized using a photo crosslink able resins like polyurethane, acrylic polymers like polyacrylamide they are all very good. So, these are monomers and then, we can use U V to create a or initiate the polymerization reaction, we can use glutaraldehyde for cross-linking enzymes. So, glutaraldehyde is also a very good chemical for creating cross-linking between enzyme.

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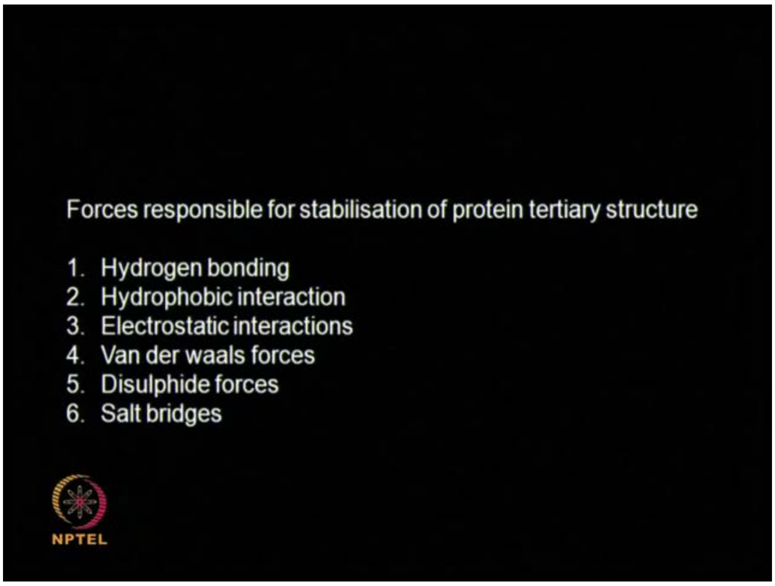

So, what are the forces that maintain the tertiary structure of a protein. So, you need to know these forces, like hydrogen bonding, hydrophobic interaction, electrostatic interaction, Van der waals forces, disulphide forces, salt bridges and so on. So, hydrogen bonding you can have n h groups or o h groups. So, the h in those o h or n h can form a hydrogen bond with the oxygen or a nitrogen present. So, the energy values are very low in hydrogen bonding, but they really maintain or keep the three-dimensional structure or the tertiary structure of the protein.

Hydrophobic interactions, like benzene rings or a long chain hydrocarbons, these contribute towards the hydrophobic interaction. Then we have the electrostatic interaction; that means, the atoms create an electrostatic repulsion or attraction and then the structure is maintained, then we have the Van der waals forces. Then we have the disulphide forces and finely the salt bridges, all these types of forces or interactions, help in maintaining the three dimensional structure of the proteins.

This binding energy or the values, energy values for these forces may be much less when compare to your covalent bond, but still these forces maintain the three dimensional structure of the protein and hence the activity.

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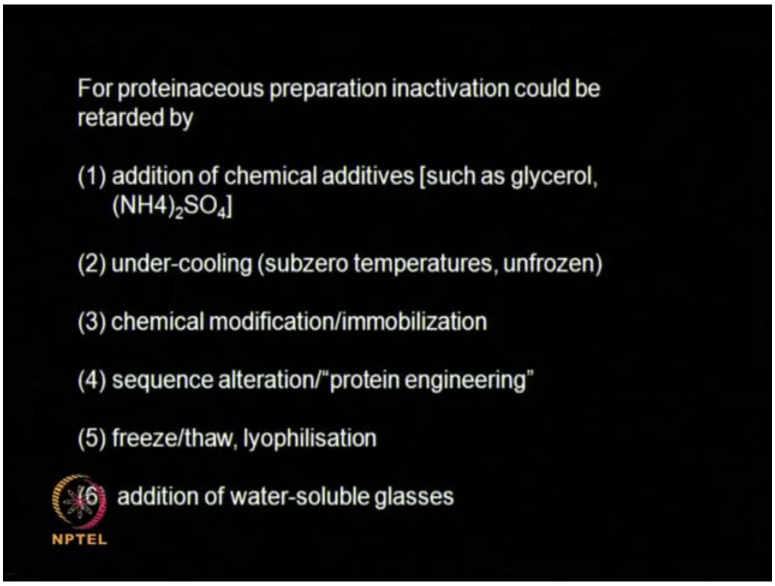

So, if you have proteinaceous material, you can retard the loss in activity, by the addition of chemical additives such as glycerol, ammonium sulphate or we can bring down the temperature, very low temperature tertiary structure sum 0 temperature or even we can make it frozen, you can even do chemical modification that is immobilization.

We can even do sequence alteration protein engineering, we can even go to freezing thawing that is lot of lyophilisation we can even think about adding water-soluble glasses. All these different approaches can help in reducing the inactivation of proteins; that means, we can retard the inactivation of protein.

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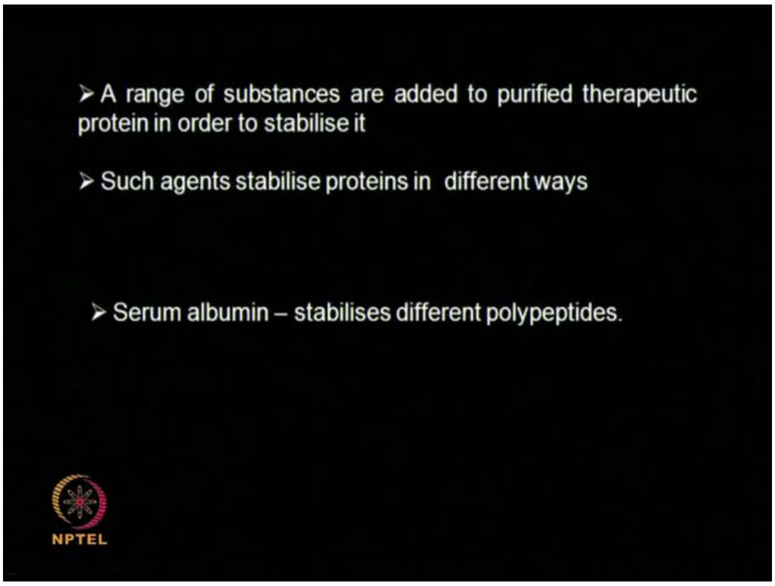

For example, if you look at therapeutic proteins, large number of substances can be added that the main problem. You need to consider if it is consumed, by human then the additives which are adding, which will prevent there a the inactivation should also be biocompatible should also be approved by the food and drug administration that is very, very important actually. Now, look at serum albumin, human serum albumin is used in many places for preventing the deactivation of a therapeutic proteins and different polypeptides.

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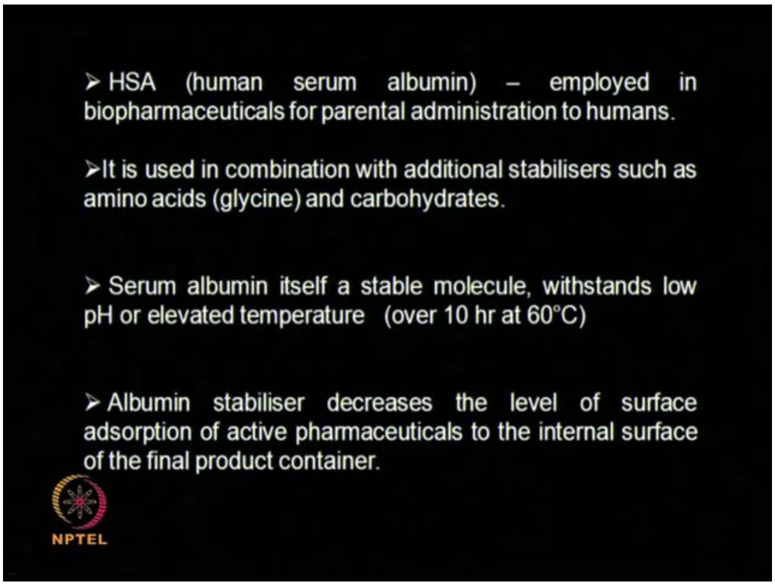

So, they are quite widely used in biopharmaceutical products, which are used for administrating to human. So, they are also used in combination with the other stabilizers such as amino acids like glycine or even carbohydrates. This serum albumin is also a very stable molecule they can withstand acidic p H or even they can even withstand high temperature almost up to 60 degree centigrade for about 10 degree 10 hours actually. So, what does it do the albumin stabilizer decreases the level of surface adsorption of active biopharmaceutical to the internal surface of the container. So, they form something like a liner inside the container.

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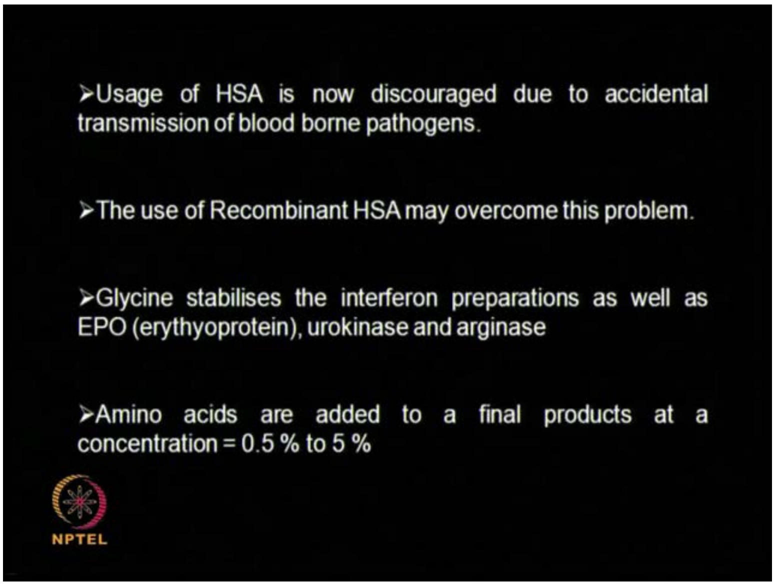

But, then there is one single problem with the human serum albumin, is now, a day is discouraged because there could be an accidental transmission of blood borne pathogen. So, you have to be very careful that the human serum albumin, which we use as a stabilizer do not have any blood borne pathogen, but then new approach are being adopted looking a recombinant human serum albumin. So, that we do not directly use the human serum albumin from human blood, but we can think about recombinant HSA. So, this can overcome the problem of transmission of blood borne pathogens.

We can even think about using glycine because glycine stabilizers interferon preparation as well as it can help in stabilizing, erythyoprotein, urokinase, arginase. So, large number of enzymes can be stabilized by adding glycine. Even amino other amino acids can be added to a final product concentration ranging from almost 5 percent, starting from about

0.5 percent. So, many amino acids are added for stabilizing many of these enzymes actually.

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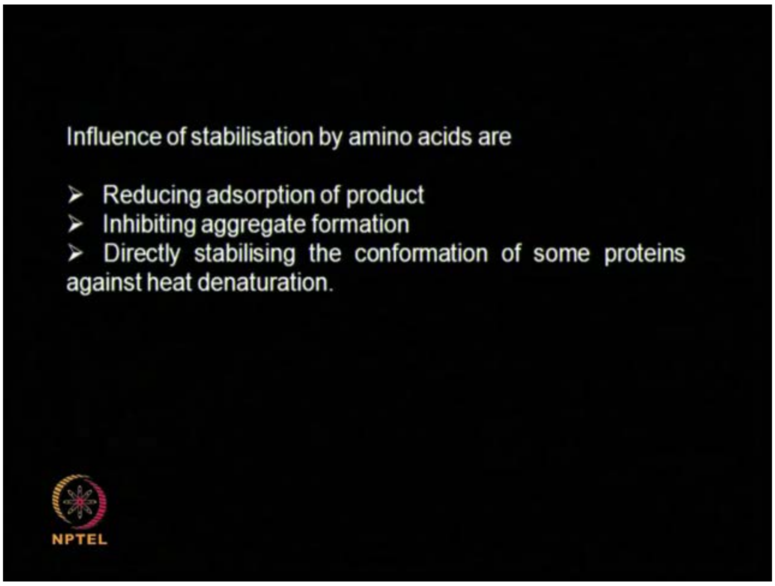

So, what does it do, it reduces the adsorption of the product, it inhibits aggregation of the formation it also stabilizes the conformation of some proteins against heat denaturation. So, because of temperature if denaturation happens addition of these amino acids good retard this type of a reaction.

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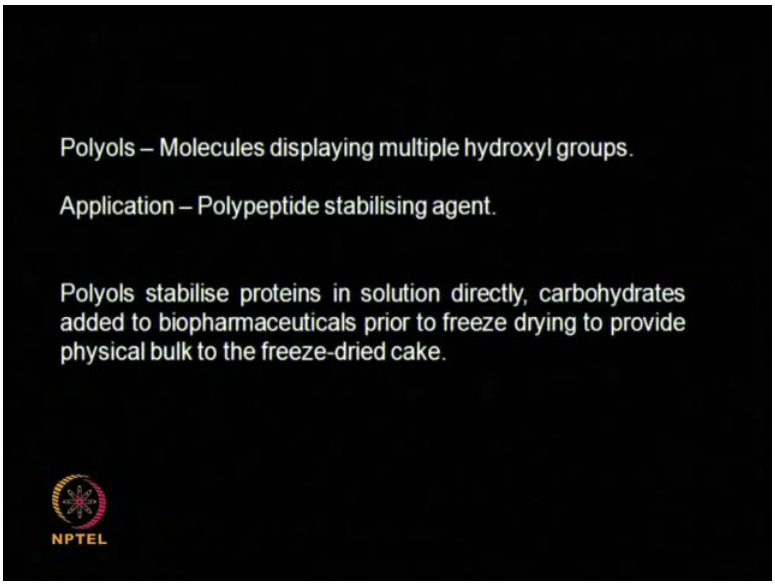

Polyols; that means, molecule which have several hydroxyls, they are also very good stabilizing agents because of the many hydroxyl. So, polyols stabilizes proteins in solutions directly, then we can also had carbohydrates to biopharmaceuticals prior to freeze drying, by adding these it provides a physical bulk to the freeze-dried cake. So, when it is dried, the cake looks big or bulk here because of the addition of polyols. So, they are used in application for stabilizing polypeptides.

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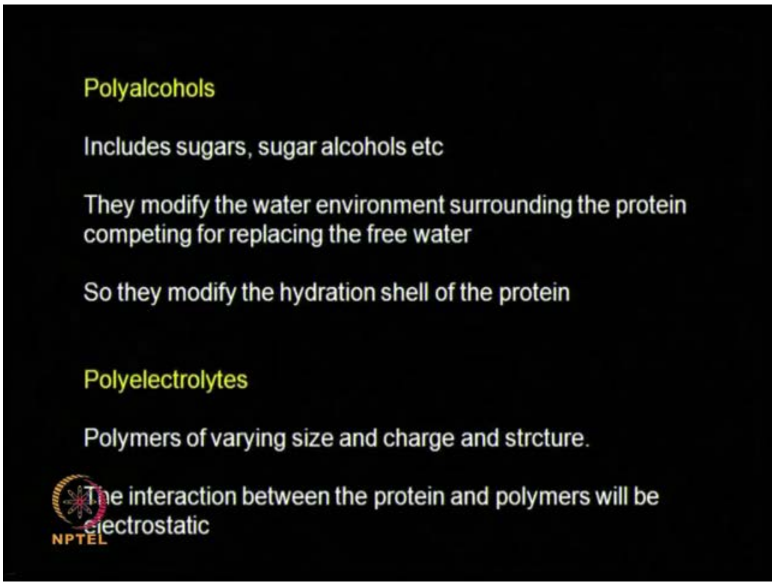

So, the polyols or polyalcohol then include sugar, sugar alcohols etcetera. So, what do they do because they are highly hydrophilic or polar in nature, they modify the water environment surrounding the protein; that means, the water that is present around the protein, gets disturbed by this addition of polyols. So, they replace or they surround the protein and move the free water away from the protein. So, they are they affect the hydration shell of the protein.

So, when you add polyols the hydrations say shell is disturbed, the free water that is present near the protein which may contribute towards the destabilization of these proteins and so on. You can even add polyelectrolyte's. What is polyelectrolyte's? Polyelectrolyte's are nothing, but polymers of varying sizes they have charges. So, what do they do, there is an interaction between these polyelectrolyte's and the protein and because of this charges there is a stability of the three-dimensional structure of the protein. What is interaction, that is electrostatic interaction between these polymers and the protein. So, polyelectrolyte's is another approach by which, we can stabilize the protein three-dimensional structure.

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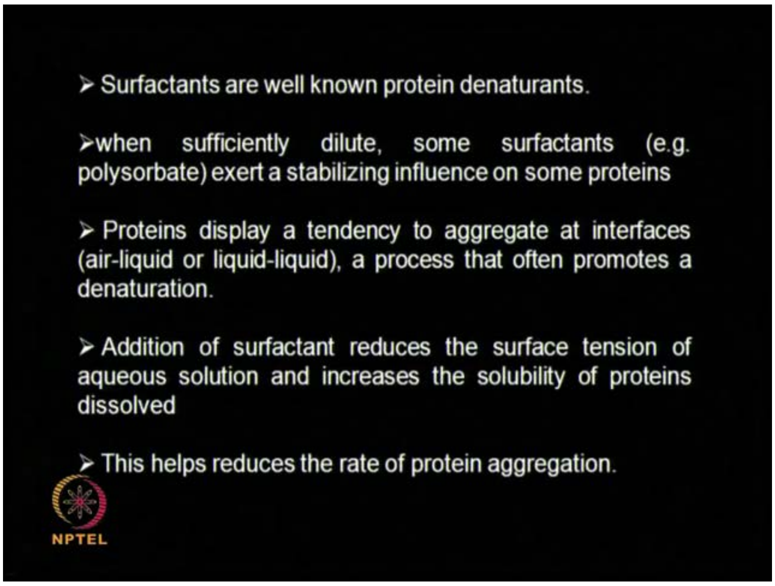

Now, if you look at surfactants. Surfactants if they are added in large number, large amount they denature the protein, but if you have very dilute for example, like polysorbate, they stabilize the three-dimensional structure of the protein. Generally, what happens if you look at protein they aggregate at interfaces, whether it is air-liquid or liquid-liquid interface. Once, they start aggregating near this interface, what happens they denature because they aggregate and then get denature.

So, whereas, if you add little amount of surfactant. What happens the surface tension of the water is reduced. So, the proteins get solubilized. So, they do not go to the surface and aggregate, there by you can prevent the denaturation of the protein. So, but you need to add small amounts because large amount of surfactant may affect the protein three-dimensional structure, small amount disturbs the surface tension and solubilizes the protein there by reduces the amount of protein present, at the air-liquid or liquid-liquid interface. When more protein goes at air-liquid or liquid-liquid interface, they aggregate. Once they aggregate they lose their activity they gets destabilized.

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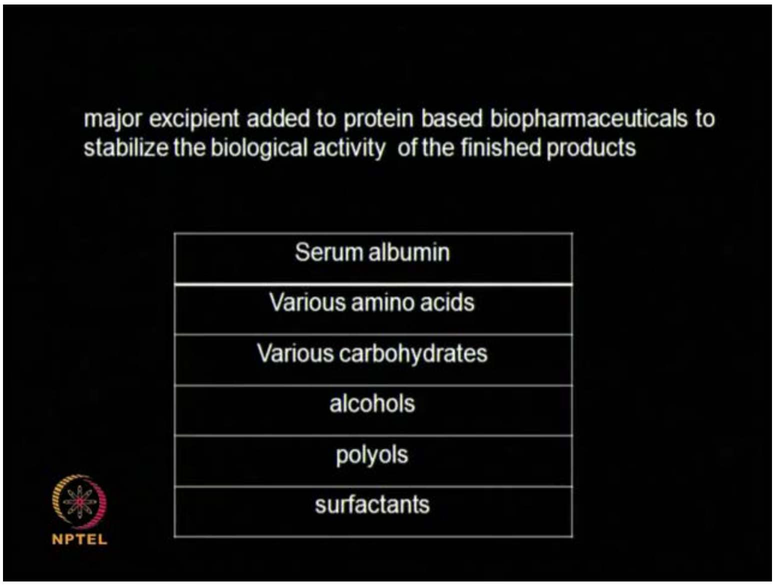

So, what are major excipient added to protein based biopharmaceuticals. Serum albumin, like human serum albumin, various amino acids, various carbohydrates, alcohols, polyols; that means, sugar which have many hydroxyl groups even some amount of surfactants. So, all these are added for stabilizing protein based biopharmaceuticals.

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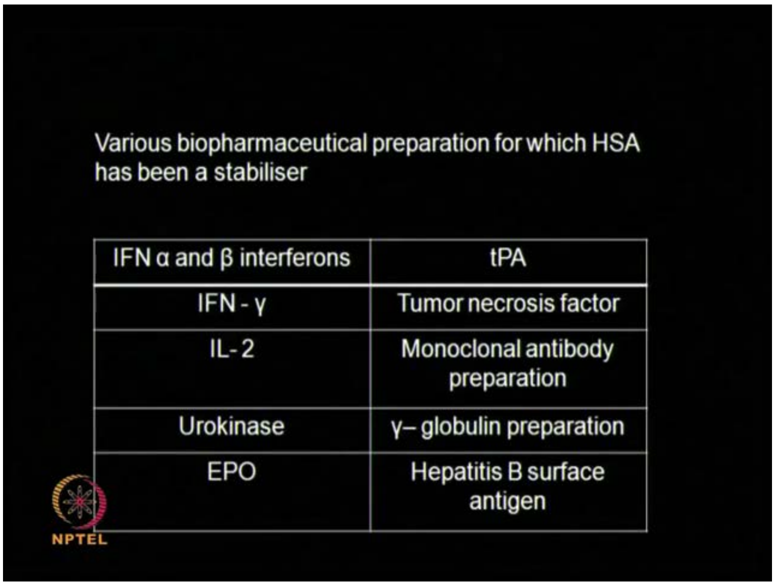

Especially, where do you add a human serum albumin, again it is used in some several biopharmaceutical preparations like, if you are preparing IFN alpha and beta interferon's

IFN gamma IL-2 urokinase ento protein all these products could be stabilized by adding human serum albumin.

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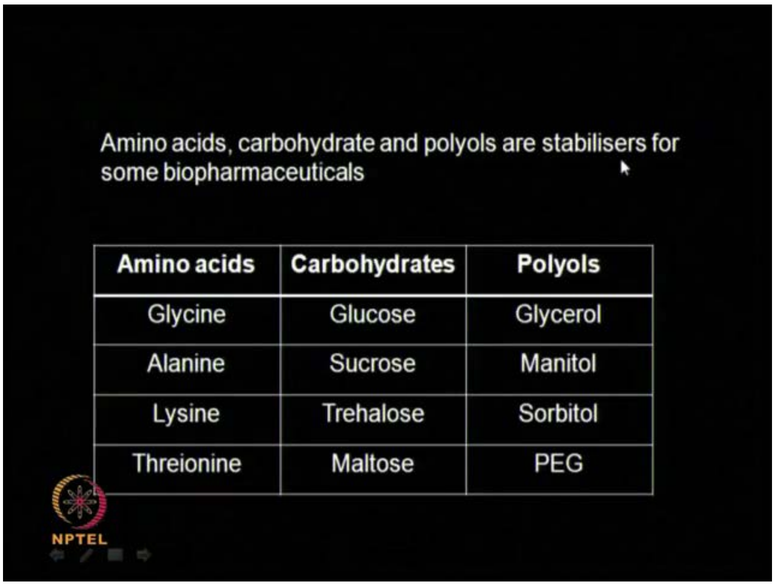

Again, amino acids, carbohydrates, polyols are widely used for biopharmaceutical products, like glycine, alanine, lysine, threionine, they these are the amino acids which are used in stabilizing biopharmaceutical products, glucose, sucrose, trehalose, maltose, these are the carbohydrates used polyols like glycerol, manitol, sorbitol, PEG of various molecular weights are added for stabilizing biopharmaceutical products. So, you can choose a large number of amino acids, carbohydrates, polyols which are biocompatible which can be consumed by human, which are widely used this telepathic application as well as biopharmaceutical application.

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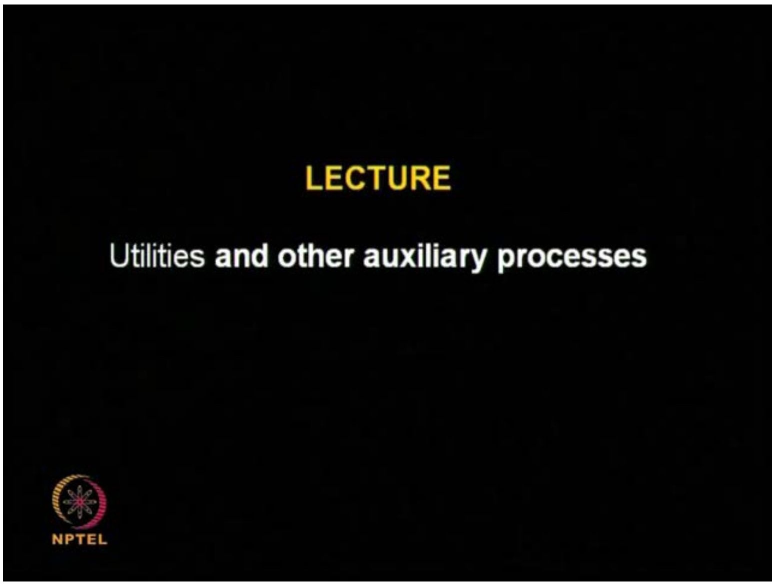

So, we talked quite a lot about stabilization. Now, let us go to another topic that is called the utilities and other auxiliary processes see, here a bioprocess manufacturing plan, does its not only contain your bioreactor or fermentor and downstream, but it also contains lot of auxiliary material in a storage vessels, heating systems, steam, hot oil, compressed air, chilling planned, even effluent treatment plan all these are also part of your manufacturing facility.

And I would like to briefly mention all these topics as well because a manufacturing facility is not only made up of only the reactors and the purification vessels, but it also contains large number of auxiliary units, as well as utility units. And one needs to know how complicated a manufacturing plant is, if one understands everything about a your manufacturing facility. Now, what are the utilities that are normally used in a downstream process, large number of utilities depending upon the nature of downstream. Some of them I am going to briefly talk about.

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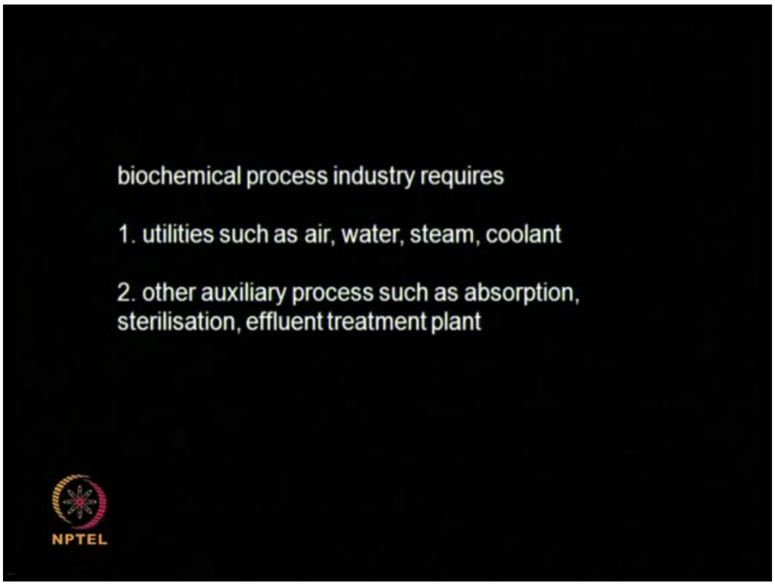

So, a biochemical process industry requires air, water, steam, coolant, chilled water, brine, hot water, a high pressure steam, nitrogen and so on these are called the utilities. And then there are other auxiliary processes such as, absorption because you may be producing toxic gas, you may be producing and dangerous vapors, which cannot be just wended out into the atmosphere.

So, they need to be absorbed before, the inert gasses are wented out to the atmosphere you need to have sterilization facilities, and then you need to have effluent treatment plant, you again once again cannot went out, solids, effluent or liquid effluent or gases effluent, without doing certain amount of treatment. So, that it becomes harmless. So, these are the auxiliary processes that are normally seen or normally part of your manufacturing facility. So, we will talk about these as well very briefly.

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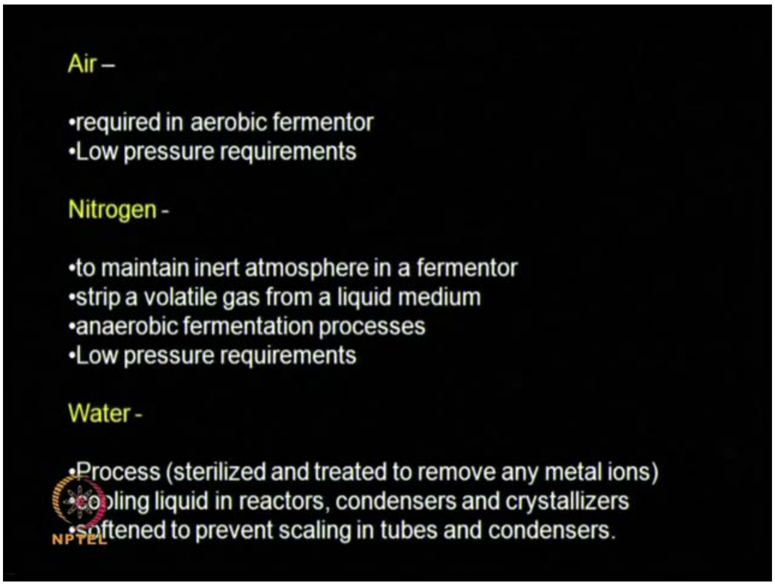

Air. So, where do you use air, air is required in aerobic fermentor. So, we need to have your facility for preparing getting air, compressing air, purifying air, sterilizing air because in an aerobic fermentor we cannot just introduce air directly, from the atmosphere we need to sterilize. So, that all the bacteria or fungus or killed other dirt and other impurities, airborne impurities are removed. So, you will require a filtration and then you will require some sort of a sterilization.

Air is also required, in filtration or some other operations as well. So, it is not only fermentor, but some other downstream as well, Now, look at nitrogen, why do you require nitrogen, if you want a maintain inert atmosphere for example, if I have hydrocarbons, present and which may be flammable I want a maintain inert atmosphere then I use nitrogen. I want strip or remove a volatile vapor from a liquid medium I want nitrogen because I will bubble nitrogen trade.

When I am doing anaerobic fermentation I do not want any oxygen present inside my fermentor, then I will use nitrogen. And sometimes I am using vacuum and I want to bleed, instead of bleeding air I am bleed nitrogen inside. So, in many situations you will require nitrogen. Water, water is needed for process, water is need for cooling your liquid in reactors, cooling liquids in condensers, cooling liquids in crystallizers. Water is needed as a process in fermentor right.

So, because you are producing the fermentation broth you where you are having a carbon source, nitrogen source, and nutrients and salts and metals and so on. So, all these are dissolved in water that is a process water. So, that water has to completely sterilize, softened and it should be free of any other impurities metal ions impurity. So, lot water free treatment has to be done, if it has to be used as a process water.

If you do not remove the metal ions even if you are using the water in condenser, it may forms scales in the tubes of the heat exchanger. it may forms scales in the tubes of the condenser which may affect the heat exchanger or condenser for a long period of time. So, you need to softened the water, you cannot directly use the municipal water, but you need to softened the water for that, but if the water is going to be used as a process water, there has to be lot of preprocessing before it can be used because it should not have any metallic impurities, it should not have any bacterial or fungal, growth it should not have any other salts presents inside that is with water.

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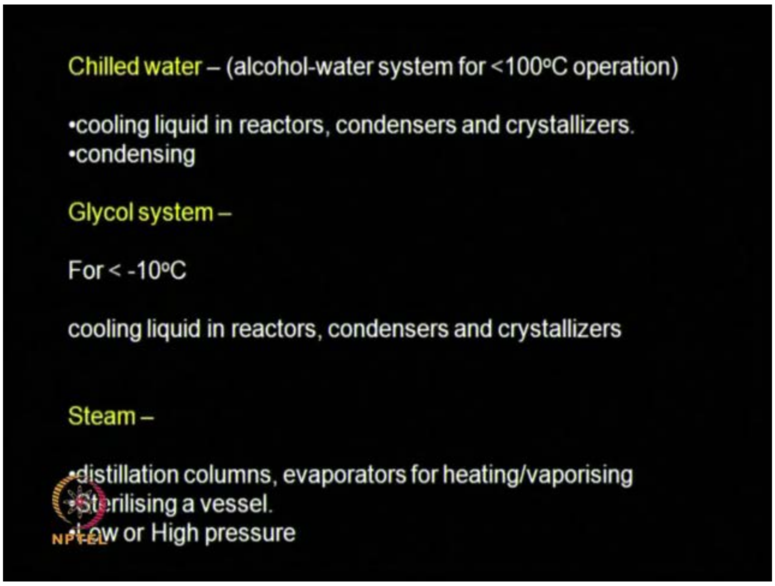

Chilled water. When do you use chilled water, you use chilled water if you want to cool the reactors, condensers and crystallizers. Sometimes, we even use alcohol water mixture if you want achieve very low temperature, like 5 degrees, 10 degrees, but otherwise if you are talking about 40, 50, 60 degrees then we can use normal water, but these water you need to recycle because you cannot throw it out, water is very expensive then free treating the water also adds to the cost.

So, all these water used in condenser, heat exchanger, crystallizers, have to be recycle, but still there will be some lost during the recycling operation. So, chilled water is used in cooling reactors, condensers, crystallizer and so on actually. If you want to achieve very low temperatures that is below 0 degrees, we cannot use chilled water, we may have to glycol, where you can go very low temperature minus 10 degrees less than that. So, this can be used again in reactors, condensers, crystallizers, depending upon the type of operation.

If you are talking about your lypholizer I want to achieve very low temperature I because want to convert water liquid water into ice. So, I need to use water at minus 10 or minus 15 degree centigrade. Now, steam, steam is used for heating, in distillation columns in evaporators for heating vaporizing, sterilizing a vessel because most of the reactors, before startup has to be completely sterilized. So, there we use steam, the steam we can use low pressure steam or high pressure steam.

So, up to 130, 140 degree centigrade you may be able to use low pressure steam, if you want to 150,160 degree centigrade you go to high pressure steam. So, distillation column may require high pressure steam whereas, your sterilization, heating the reactor or columns or other vessels or sterilizing you may go for low pressure steam. So, depending upon the type of requirement your manufacturing plant may have a boiler making a low pressure steam boiler or a high pressure steam boiler, and depending upon the requirement quantity of requirement the boiler size also will vary.

So, steam is very, very important and all the downstream sections, will definitely have a steam manufacturing plant.

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Hot oil. If you want very high temperature then hot oil is always preferred 180, 190 degrees rather than steam, if you are heating a distillation columns, if you are evaporating, sterilizing vessel. Advantage of steam is you get very good heat you do not need to recycle the steam will transfer the heat and at get condense as water which could be correctly covered whereas, if you are using hot oil, the hot oil written line is very, very important. So, you have hot oil going to the plant and then a cold oil coming back to the heating system.

So, expenses are very high. Heat transfer is very high with steam. So, steam is preferred there, but still if you are talking about 200 or up then instead of going for a high pressure steam boiler, you may go for a hot oil system. So, these are some of the very important utilities in manufacturing plant. The air may be the nitrogen, steam then cooling water, sometimes chilled water or even glycol if you are trying to go in temperature. So, all bioprocess manufacturing facility will have few of these utilities.

So, you need to understand how much utility will require for the entire plant, what is the rate of utility requirement and what is the temperature desired in various places in the plant. So, because they will all add up to the capital cost as well as, they will all add up to the manufacturing or running cost of the plant. And sometimes, the utility cost add up to about 12 percent of your manufacturing cost, in addition to raw material. So, please remember that.

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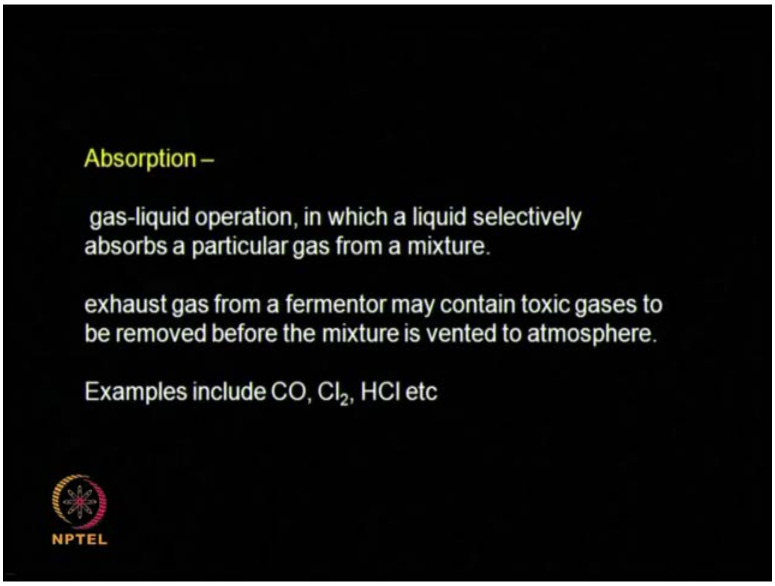

Now, there is another down auxiliary unit that is called absorption. Generally, the vapors coming out of your fermentor may have many toxic, gases, harmful gases which need cannot be wanted; for example, you may have CO coming out, you may have chlorine coming out, you may have HCL coming out, you may have SO 2 coming out you may have some hydrocarbons coming out.

So, you might not like to just went it out into the atmosphere because they could be harmful to the surrounding to the human as well as animals and even the ecology. So, what you do, you may have to absorb these toxic gases and went out the non-toxic ones the inert ones. So, there you need to go for an absorber. So, absorber; that means, you need to design an absorber, you need to have a running an absorber which will again add to the running cost.

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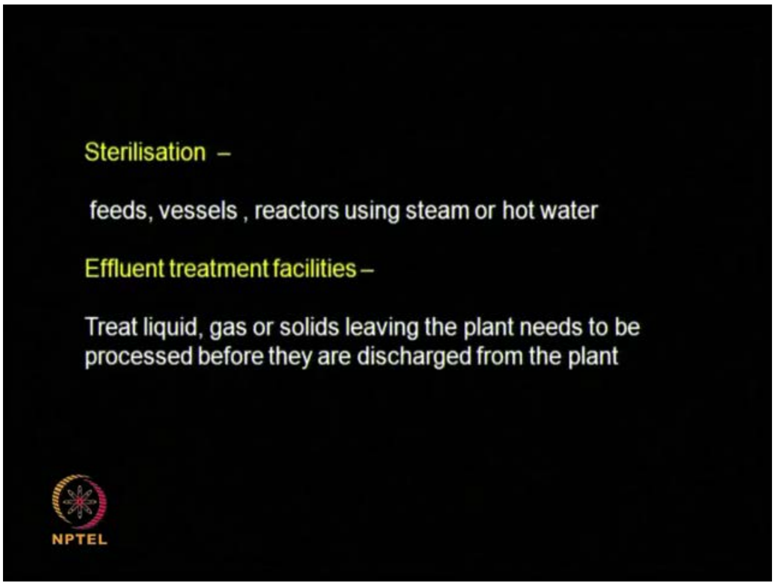

Then you will require sterilizing. Most all the feed, liquid feed that is entering the fermentor or a bioreactor has to be completely sterilized. All your feed vessels have to be sterilized before starting a batch or a campaign, your reactors have to be sterilized all the vessels, holding vessels, have to be sterilized. So, you need sterilization, facilities; that means, you use hot water or steam to do the job.

And then effluent treatment plant, as I said you cannot just dispose of the gases, liquids, solids coming out of the plant into the municipal way because they could be very toxic, they could be harmful to the flora fauna, human to the public and so on. So, they need to be treated. So, your manufacturing plant, will also have an effluent treatment facility, in built and the liquid or the solid or the gaseous affluent has to be treated and dispose as per the norms of the local pollution board.

So, the local pollution board norms may change from country to country, region to region and where you are disposing if you are disposing it into a river, they may have some norms, if they are disposing it into the sea, they may have some other norms or if they are disposing it to the ground water, then they may have some other norms. And then solids, if you are very close to the sea, you may have some other norms for disposing, whereas if it is inland you may have some other norms.

So, the effluent treatment facilities also are very, very important because they have add to the capital cost as well as, to the operating cost because you have to continuously run the effluent treatment, to process the effluent that you generate in your plant where do you get effluent, you get effluent from your fermentor the dead biomass, salts, unwanted metabolites you get effluent from distillation columns, you will get effluent during extraction the unwanted material, you get effluents from your filtration, solid material you will get effluent from some other chromatography, after you are recover the desired product.

So, you will get effluent from each stage of the downstream process. So, different types of effluent treatment techniques are available, depending upon the type of effluent and the quantity of effluent and the seriousness of this effluent. A typical liquid effluent treatment plant, this is how it will look like a typical because it may have more units it may have less units depending upon the characteristics of the effluent, for example.

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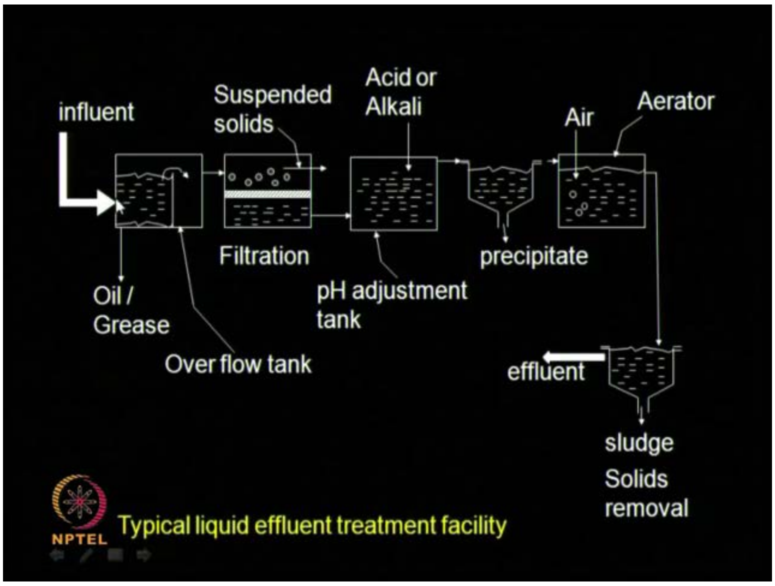

This is your influent, just coming out from the rest of the plant. First step may be removal of oils and grease. So, oils and grease may be in the form of a emulsion. So, they may settle down here, and the overflow will be free from the oils and grease. So, continuously you may withdraw from the bottom the oils and grease. So, what does doing the oil and grease will agglomerate and because they are heavier they will settle down here.

Then next step you may have a filtration. All the solids I may be retained and the liquid part may be coming down. So, different types of filtration may talked about can be even adopted here, then the effluent may be very acidic or alkaline. So, you need to neutralized the adding an acid or an alkali here, and then you may be because there is an acid or alkali added there could be a precipitation taking place, the solid will come down as precipitate now, the liquid, neutralize liquid which is free from oils and grease may come to your aeration tank.

Sometimes you also have a flocculation taking place; that means, you add flocculent to remove the some of the product which is not settling down. So, there could be a flocculating tank also in between. Once all these solids, we suspended solids and the dissolved solids have been separated it goes into a aerator. So, you are bubbling air into it and you may add some activated biomass here. So, there is a degradation of this material taking place, this the residence time could be in the order days it may be 4 or 5 days.

So, once you do that, there is a sludge, which is removed and the effluent comes out which can now, be discharged into your municipal open ways. So, you are removing the suspended solid, you are removing the dissolved solids and then you are degrading the material using air and biomass. So, this is a typical liquid effluent there, could be a many modifications depend upon the nature of effluent.

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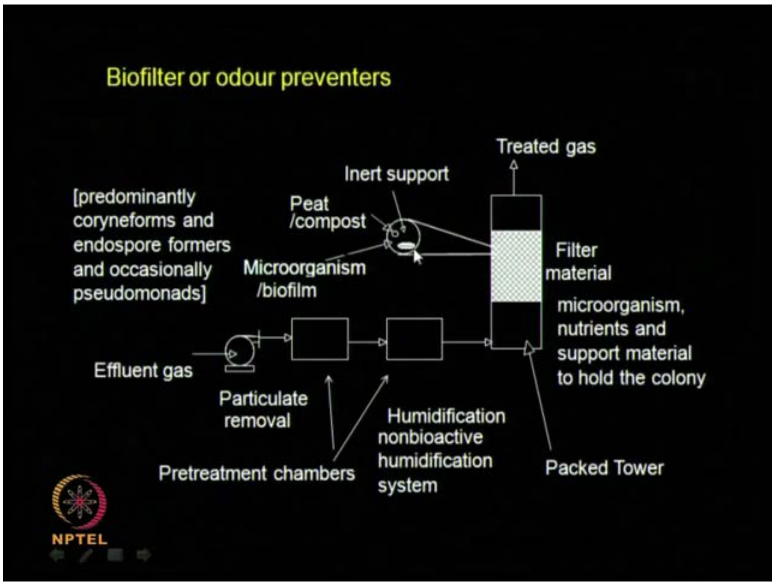

If you have gaseous effluent what you do, we can use biofilter or it is called odour preventer. So, a biofilter is nothing, but a tall column, you are packed this column with filter material, this material could be inert support, and then it could be a nutrient like peat or compost and then you put in some micro organism or biofilm. So, what happens this microorganism grows because of the presence of this carbon source, and inert support acts as a support.

So, when a gas containing, unwanted material or unwanted gaseous material they get degraded by or biodegraded by the microorganisms and the treated gas comes out which can be wented into the atmosphere. So, the effluent gas initially, is filtered to remove all the particles and then you may had humidification or that means, you spray little bit of a moisture. So, that the gas is bit wet and then when it travels through the column, which contains microorganism, the microorganism degrades the unwanted gas. So, the treated gas, comes out which is free from that gas, unwanted gas this is called a biofilter or a odour preventer.

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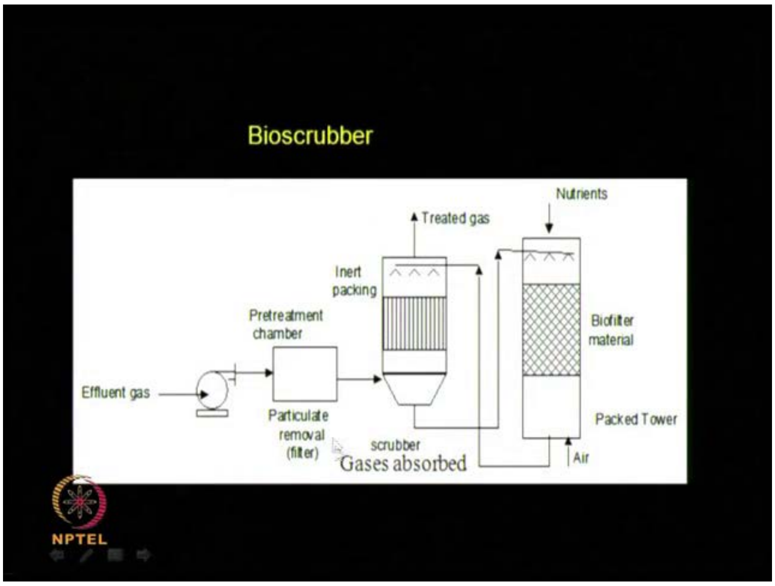

You can have another type of design that is called a bioscrubber. So, what does it do you have a scrubber and you have a biofilter combination. So, you have the effluent gas coming in initial filtration may be taking place, and once you have filtered it goes through the scrubber, water is spread of the top. So, the water dissolves the gas which needs to be scrubbed, it dissolves the unwanted gases only they are treated or inert gas escapes from the top of this scrubber.

Now, this liquid which contains the gas that needs to be degraded, flows through your biofilter you also add nutrients. So, the solid material is the place where the microorganism is growing, when it comes in contact with this liquid effluent it degrades the unwanted material, you also pumping air from the bottom. So, that there is a aerobic type of degradation taking place here, this is called a bioscrubber.

So, because it contains a scrubber where the gas, flows from the bottom and water is spread from the top and it scrubs this particular gas free of the unwanted odours or unwanted vapors or unwanted gases, and these are degraded in the biofilter which is here, located here. So, this is a combination of both the scrubber as well as the filter. So, this is another design for removing or treating gaseous effluents.

So, these are few designs which I have shown, but that does not mean it is exhaustive because our focus is not on biological treatment or biological degradation of gaseous or liquid effluents are focuses more on the downstream, but I had introduced these topics because I wanted to emphasize a manufacturing plant is not only made up of only bioreactors or fermentors or purification systems, but it also contains many other systems, like utilities other auxiliary units which are also very, very important, which also form part of your manufacturing plant.

And they also contribute towards the capital cost and they also contribute towards the operating cost. And now, a day’s with the effluent treatment becoming very stringent, treatment of the effluent adds to the overalls operating cost in the manufacture of a particular biological product. So, if the treatment cost is very high, your selling price also will go up, if you need a very sophisticated treatment plant then your capital cost for the manufacturing plant also goes up.

So; that means, your product becomes more expensive. So, in order to understand that you need to have some idea about the auxiliary units that are present in your manufacturing plant. We mention that a scrubber is a very, very important part, in your manufacturing plant because the gases that are leaving a fermenter or a bioreactor cannot be directly wented out because it may have carbon monoxide or sulphur dioxide or chlorine or hydrocarbons or other toxic chemicals, which may be harmful to the flora fauna.

So, they need to be captured or observed. So, generally the absorption is done with the help of a liquid which absorbs these gases. So, absorption process is a gas liquid operation. So, I would like to spent here few slides on the concept of absorption.

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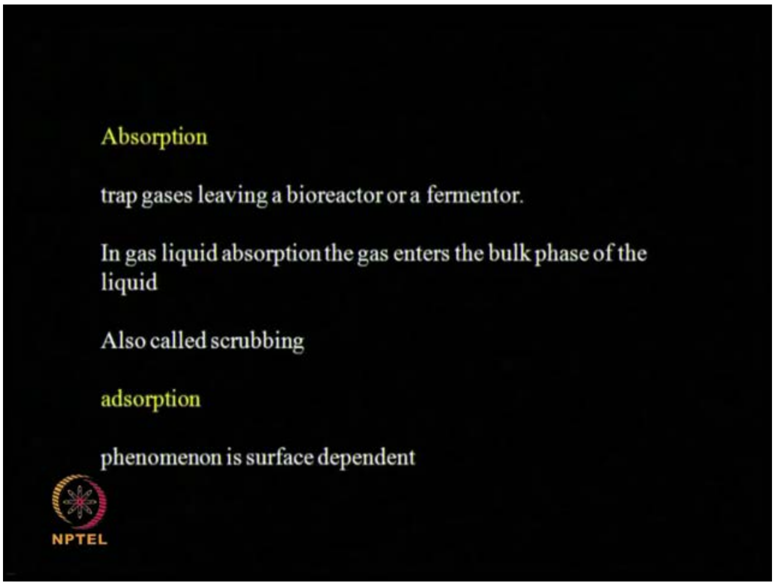

So, what is absorption or why it is done, it is done to trap gases leaving a bioreactor or a fermentor. So, in a gas liquid absorption, the gas enters the bulk phase of the liquid and certain gases are selectively absorbed, it is also called scrubbing. Whereas, in adsorption ad it is a surface phenomenon the gas and the solid interact at the surface or the interface between the solid and the gas. Whereas, in an absorption that is a b there is an interaction with the bulk face of the liquid. So, generally absorption is bulk phenomena, interaction between gas and liquid, adsorption is a surface phenomena it is an interaction between the gas and the solid.

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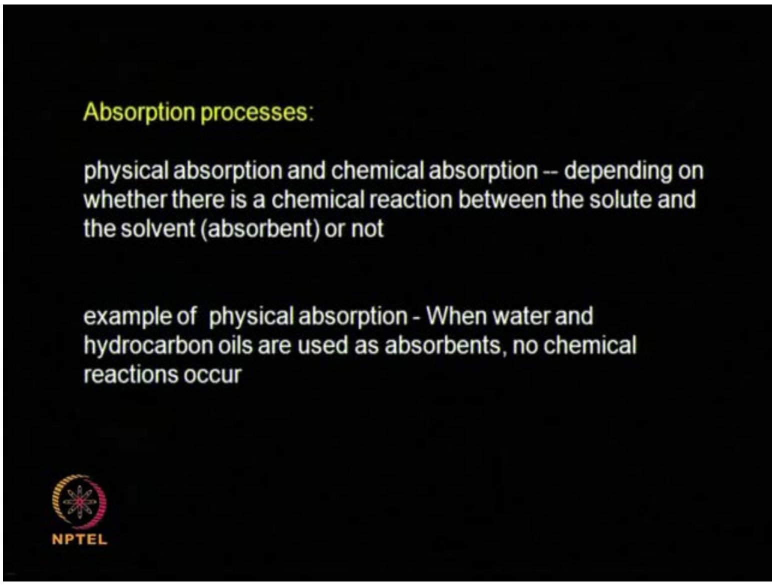

So, there are two types of absorption, one is called the physical absorption, other is called chemical absorption. So, depending upon whether there is a chemical reaction that happens after the absorption, if there is an absorption followed by a chemical reaction then it is called chemical absorption or chemis option. Whereas, if there is only absorption then it is called physical absorption.

So, examples of physical absorption. when water and hydrocarbon oils are used as absorbents there is no chemical reaction for example, if a take a gas. and if the a liquid is water then gas is just getting dissolved and that is called a physical absorption.

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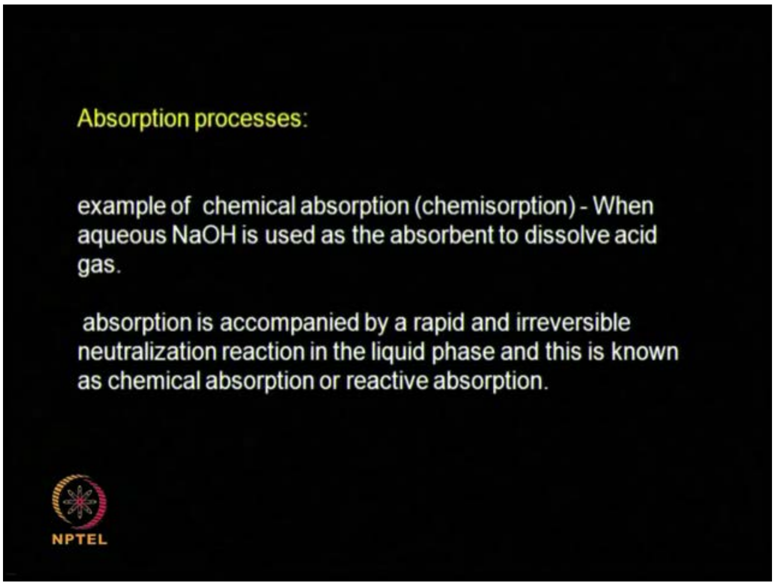

What is a chemical absorption for example, if I am using sodium hydroxide and I am using to capture, certain acidic vapor or carbon dioxide then what happens the carbon dioxide not only dissolves in sodium hydroxide, but it also reacts in sodium hydroxide, sodium bicarbonate is formed that is a chemical absorption or if I have a dilute acid vapor, they dissolve in sodium hydroxide they also react. So, that is called a chemical absorption. So, absorption is accompanied by a rapid and irreversible neutralization reaction. So, that is called a chemical absorption or reactive absorption.

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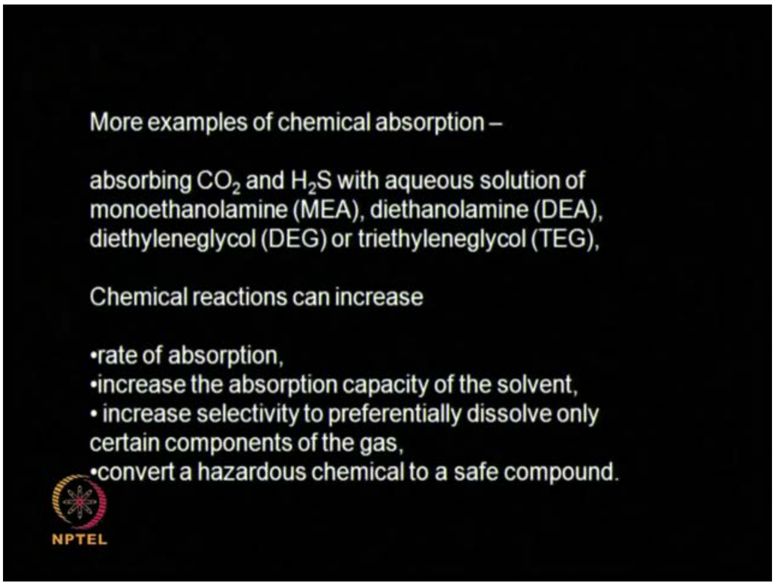

So, more of examples absorption of CO 2 and hydrogen sulfide with mono ethanolamine or die ethanolamine or die thyleneglycol or tri ethylenelycol. So, there is a absorption and a reaction. So, what is a advantage the rate of absorption increases, it increases the absorption capacity, we can selectively or preferentially dissolve only some components, we can even convert hazardous chemical to a safe compound.

So, that is the advantage of doing chemical absorption, but the main disadvantage is, the liquid which we are using gets consumed for example, if I am using sodium hydroxide, it is become for absorbing carbon dioxide it is become sodium bicarbonate. So, I need to keep on adding whereas, in physical absorption the liquid is not consumed. So, I can keep on recycling the same liquid whereas, in the chemical absorption I am consuming the liquid.

So, I need to replace. So, I am using sodium hydroxide to absorb carbon dioxide I am getting sodium bicarbonate. So, I have to keep on adding sodium hydroxide. So, it is got lot of advantages, but there are disadvantages also. So, that is called chemical absorption.

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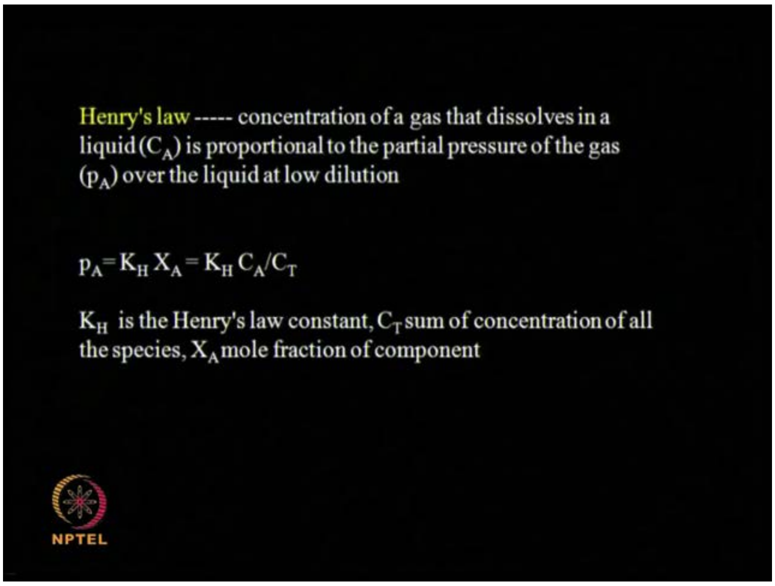

Now, in order to understand the concept of absorption, we should know something call the Henry's law. Henry's law gives us an idea about the partial pressure of the gas in the vapor, to the concentration of that particular compound, in dissolved form in the liquid. So, it relates these two parameters and Henry's law is a very, very important law, which gives you a relationship between these two parameters. And it is valid only for dilute solutions, if it dilute solutions or dilute concentration, the concentration is very, very high Henry's law is not valid. We will continue with Henry's law in the next class.