Aspects of Biochemical Engineering
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Lecture – 28
Immobilization of enzymes – II

Welcome back, to my course Aspects of Biochemical Engineering. In the last lecture, I tried to covered the Immobilization of Enzymes, this will be continuation of that. Now, you can remember in the last lecture I tried to define what do you mean by immobilized enzyme. Actually, immobilized enzyme means you confine the enzyme on the solid matrix that is call immobilized enzyme and it has several advantages means you can reuse the enzyme again and again and stability of the enzyme will be increase to a great extent and the purity of the product will be more.

In industry particularly it has lot of applications. Now, question come that I try to discuss in the last lecture that in this immobilized enzyme system what are the different applications we have. I told you it has application and particularly in the industrial sector, I can give the example of production of high fructose corn syrup. This is produce particularly this is used in the western country in the confectionery industry. how it is produced, they use the glucose they obtain from with hydrolysis of starch present in the corn and then pass through the immobilized glucose isomers enzyme and then glucose is converted to fructose and we know the fructose is ten times sweeter as compared to this is usually recommended for the diabetes patient.

Not only that if you look at beer making industry. One major problem with the beer making industry is the precipitation of protein, because beer is considered as the energized drink because it contains lot of protein. Now, and beer usually served under chilled conditions and we know protein if you heat it or cool it there is a possibility of precipitation of protein.

Now, if there is a precipitation of protein then there would be haziness in the beer which is not acceptable. So, what we can do we can we can have the immobilized protease enzyme photolytic enzyme column and pass the enzyme through pass the beer solution through this so that protein present in the beer can be disintegrate into the smaller protein

molecules which cannot be precipitated at a low temperature and we can get chill proof beer.

Now, divided into two different classes; one is called porous and nonporous solid matrix. Now, in case of porous we have more surface area and if more surface area is there possibility of more immobilization of more enzymes and if more enzymes are immobilize then we can expect more subset conversion. So, we can expect more product formation, am I right? And, so, question come what should be the characteristics of the solid matrix. So, this we discussed in the last lecture. Now, this lecture I shall concentrate mostly on the methods, techniques of immobilization technique what are the different techniques.

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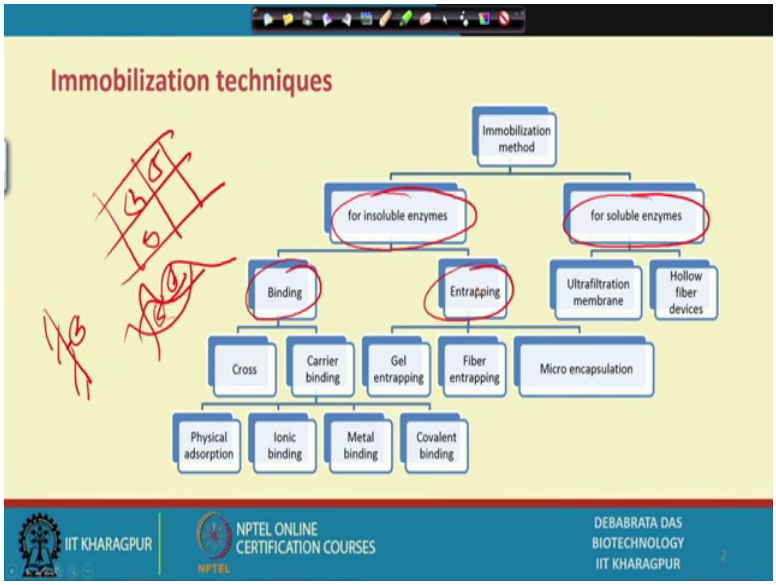

If you look at the first slide it gives the clear that you know that classification of the immobilization method. So, this immobilization method can be divided into two different way; one is called insoluble enzymes another we call soluble enzymes. Now, in case of insoluble enzymes it can be in the immobilization can be done in pass ways one by binding another by entrapping.

Now, binding means what? Binding means suppose this is the solid matrix and your enzymes is there, so, this is bind you are on the on the solid and entrapping means what? Entrapping mean suppose there is a gel inside the gel your enzyme are entrap like this,

there is a fiber you know they we have fiber like this and inside the fiber the enzymes are immobilize. So, that is called entrapping.

So, this, so, if you look at the bindings we have different types one is cross linking and another carrier binding. So, this carrier binding again divided into four different class; one is physical adsorption – physical adsorption is the very simple technique and cost involvement in this process is quite less. Ionic binding, is it through the electrostatic force of attraction it can be done and another is metal binding and covalent binding. Entrapping we have gel entrapping I have I just show you gel entrapping then fiber entrapping and micro encapsulation.

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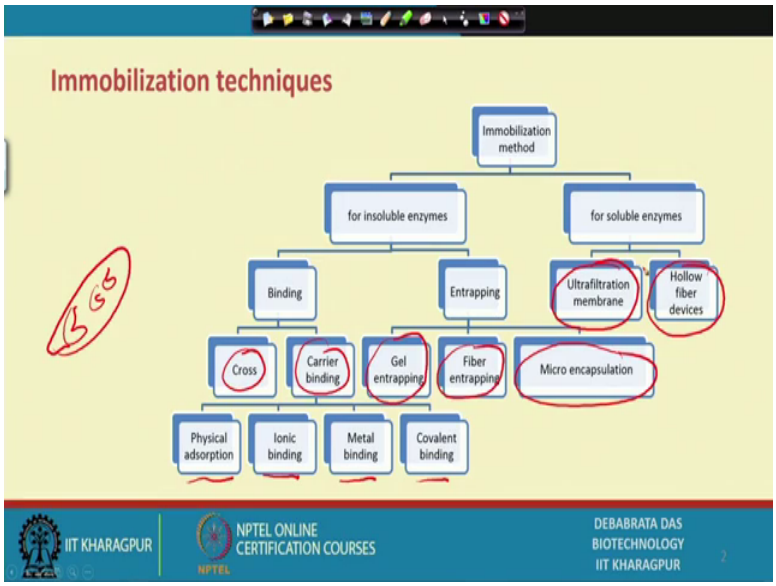

Micro encapsulation we know the envelop. Inside the envelop the enzymes are immobilize then we call it micro encapsulation very small that capsule we can put it we where I can give the example of that capsule that we use as a medicine, that you know how the medicine is put inside the capsules. Similarly, enzymes we can put inside the capsule

Now, in case of soluble enzymes the immobilization can be done in two ways one is called ultra filtration another is hollow fiber device. So, this is this is ultra filtration means it is the membrane size will be very small. So, that enzymes the adhered on the surface of the solid matrix.

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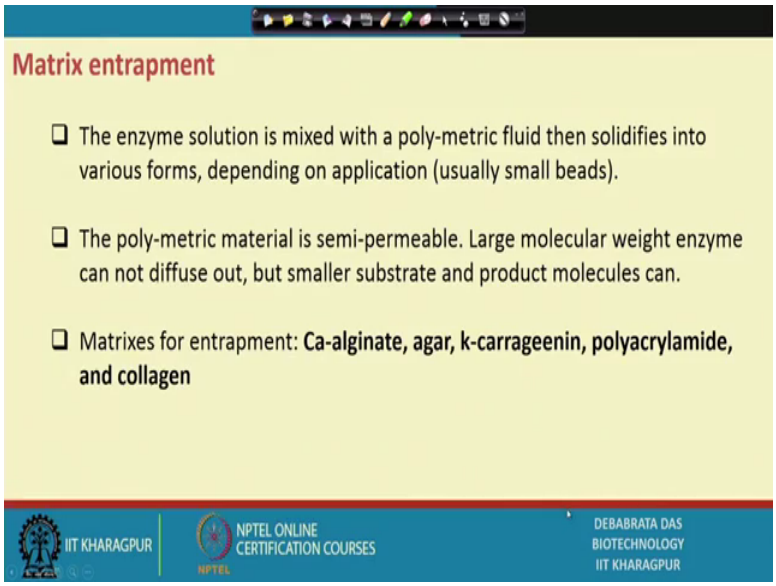

Now, matrix entrapment; now, when you talk about the matrix entrapment the enzymes solution is mixed with poly metric fluid and then solidified into various form depending on the applications. Then I can I can give the example that calcium alginate, because we know that sodium alginate solution inside the sodium alginate solution we can put some enzymes and then when you put drop by drop on the calcium chloride solution will be having bit formations that his calcium alginate. So, your enzyme will be entrap inside the bit.

Now, poly metric material or semi permeable and large molecular weight enzymes cannot be diffuse out, but smaller substance and product can be can be can diffuse in or diffuse out. Matrix entrapment we have calcium alginate, agar kappa carrageenin, then polyacrylamide and collagen.

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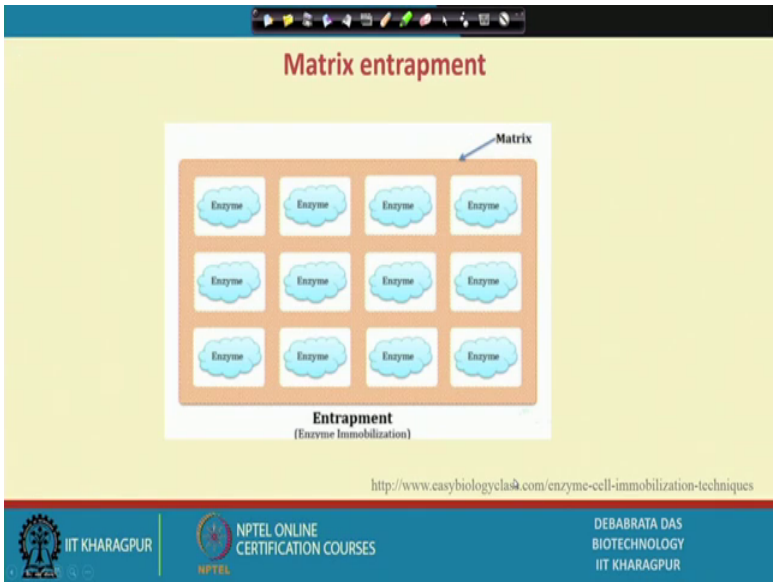

Now, this is the kind of example of matrix entrapment that I showed you this is the matrix you can see this is the matrix and inside the matrix how enzymes they are they are entrap. This is this is clearly this is this is visible.

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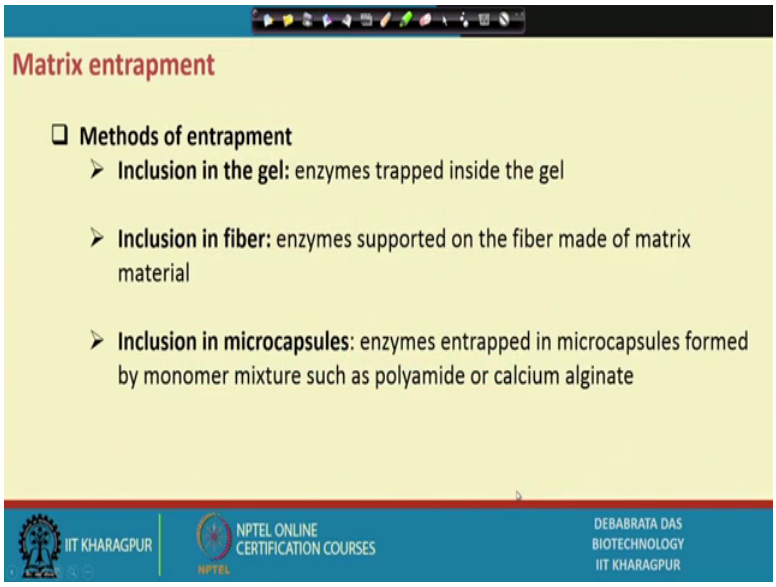

Now, then a method of entrapment since infusion in the gel, one is inclusion in the gel enzymes are entrapping inside the gel, inclusion in the fiber, inclusion in the microcapsule. I hope I have explained that, I do not like to spend more time here.

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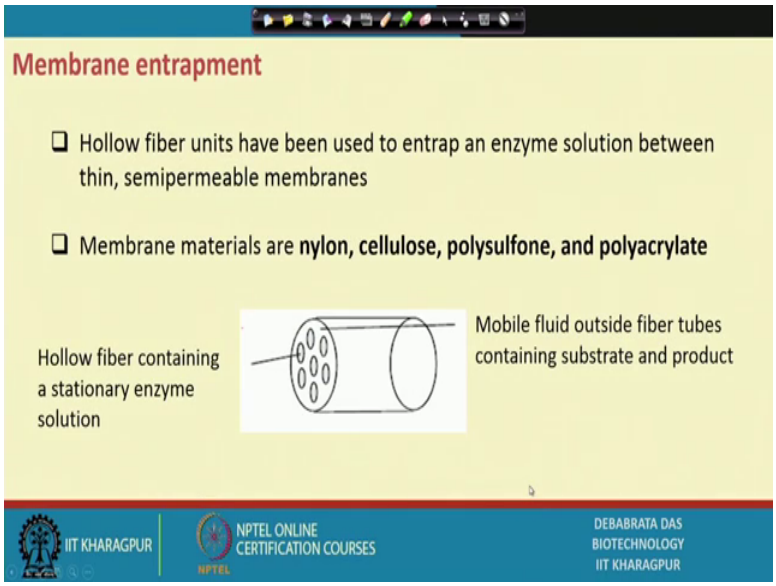

Now, membrane entrapment, I talk about that particularly in case of soluble enzyme there is a hollow fiber unit, have been used to entrap the enzyme solution between the thin and semi permeable membranes. So, you can see here the hollow fiber you know this is the hollow fiber, very small fiber and inside this fiber the enzymes are entrapped. So, this is the membrane material maybe nylon, cellulose, poly sulfone and polyacrylate, acrylate. So, different type of membranes we can use.

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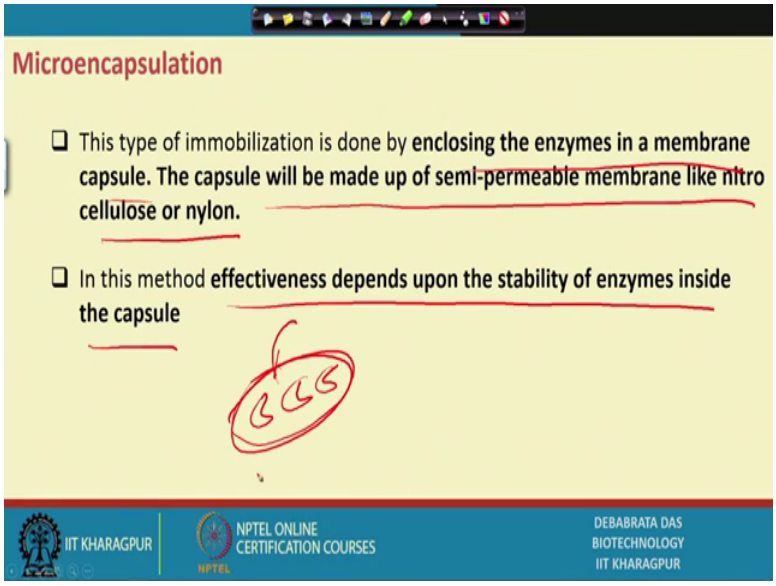

Now, microencapsulation; this type of immobilization that that enclose the enzymes in a membrane capsule the capsule should be made of semi permeable membrane like nitro cellulose or nylon in this method the effectiveness depends upon the stability of the enzyme inside the capsule. So, you know that, so, your capsule when you when you inside the enzymes that you know that now, this capsule that envelop should be stable. If it is not stable then it is break then enzyme will comes out, ordinary disintegrate the enzyme. So, stability the effectiveness of this, this capsule is very important.

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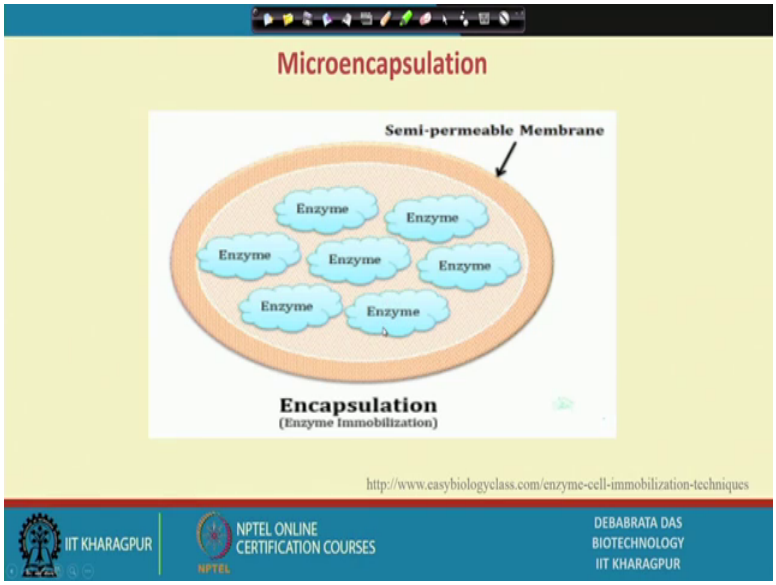

Now, this is same as what I have shown here this is pictorially how it can be shown like this.

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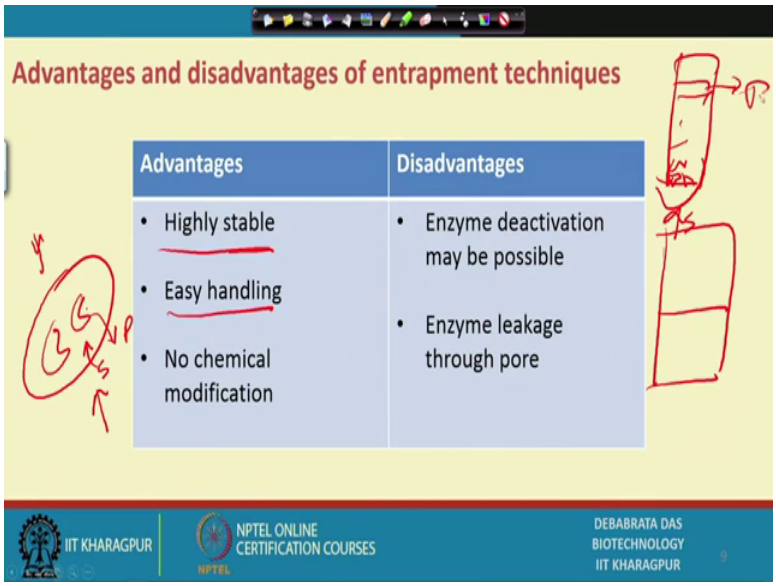

Now, what are the advantage and disadvantages of this entrapment technique? Advantage first is advantage is that that it is a highly [soluble]- stable because since it is the inside this you know that if you pass the liquid here that it is not going to going to affect the enzyme much. Only the substrate will diffuse reacts with the enzyme and product will born and product will comes out, the substrate will go in and products will comes out. Easy handling it is you can, because when I was in IIT Delhi and one of my research colleague he was working on immobilized glucose, isomerize enzyme and he is he use the collagen membrane.

The inside the collagen membrane suppose collagen membrane is a is a membrane, so, this we can make it in the form of bag you can fold it and then with the help of heat we can seal it and then one in we can put the dry powder of enzyme and seal it again. Like this we have different bag and we can suppose this is the column and of the reactor in bag we can put it here one after another and we can pack the column and then you pass your substrate here and get the product in other end.

So, easy to handle. No chemical modification is required because no interaction between the solid matrix and the enzyme. But, disadvantages that enzyme deactivation may be possible due to due to maybe your matrix has some kind of I some kind of material which affect the active site then deactivation will take place. Enzyme leakage through the

pore if the pore size of the capsule is more than enzyme may percolate out from the system.

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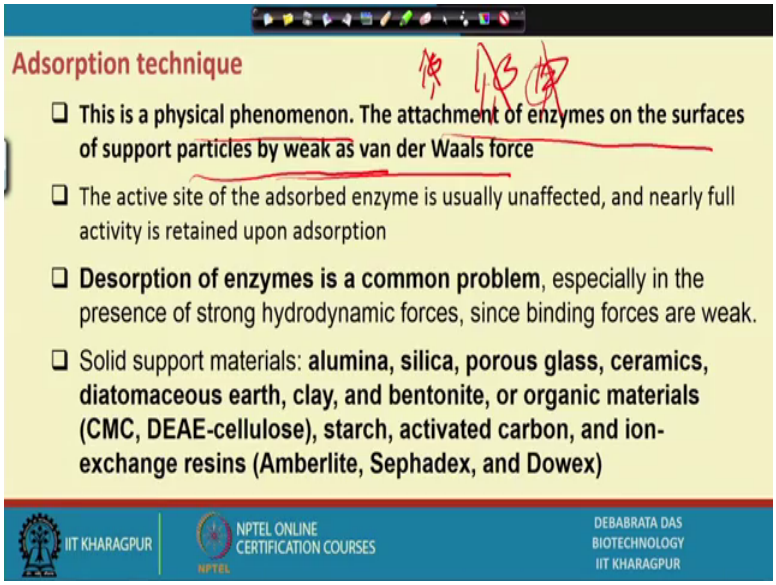

Now, adsorption technique that you know this technique largely used by the industry and this is the most simplest method of doing this, because of what we do if pack the solid matrix in the in the in the column and we pass the enzyme solution I am give a very simple example here.

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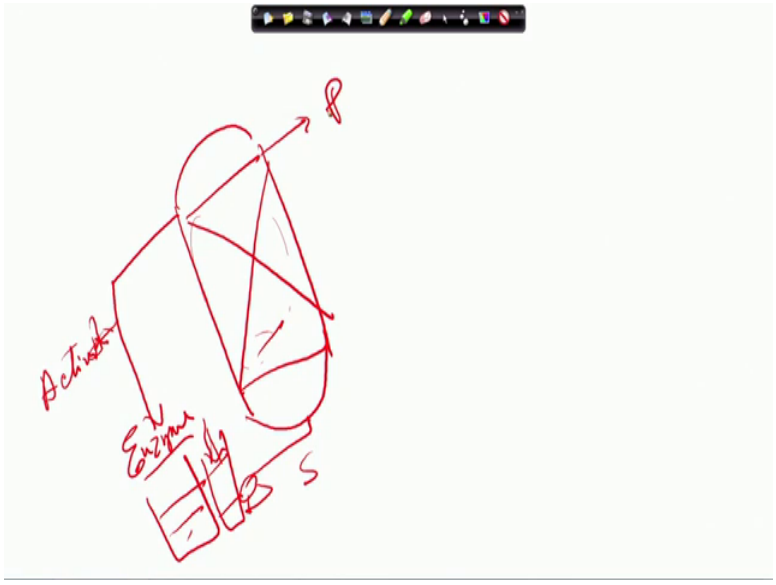

Suppose, this is a solid this is a column and inside this column we have any kind of solid matrix we can put it, we can as for example, I we can use any we can use coconut coir. Coconut coir is a fine fiber, so, we can pack the column. Why we use coconut coir because it has more lot of surface area. So, more surface area more will be the immobilization of enzyme.

Now, what you do, we prepare the enzyme suspension solution this is enzyme solution, am I right? Now, this enzyme solution with the help of pump we pass it through this and we recycle back to the system. So, we just recycle this a and time to time we draw the sample and find out the activity of the enzymes. Now, when the activity of the enzymes is constant then you assume that your column is saturated with the enzyme and then you keep it in the buffer for some time and then you pass your start you replace the with your substrate and put your substrate here and then you can take out product here very simple very easy to operate this system.

So, here the, this is the physical phenomena because here suppose this is the solid matrix. So, enzymes just the adhered on the surface of the enzyme, just adhered, you know it touches the surface and the interaction between the solid surface and enzyme it due to weak vulnerable type of force. So, if suppose if we pass the liquid at the very high flooded then it will be having some kind of shear force and with the shear force there is the every possibility can enzyme may dislodge from the surface of the solid matrix, the that is the one problem the attachment of the enzyme on the surface support if the weak by weak Van der Waals type of force.

Active site, now when we do this kind of immobilization one thing we shall have to take into account the active site of the enzyme should not should not adhered with the solid surface. Now, if it is the other way suppose this is an your active site is like this then your substrate cannot be interact with the with the enzyme.

So, you know that that is not desirable. So, that we shall have to take care then when you do the immobilization the inactive site of the enzyme should take part for the immobilization of the enzyme. Now, desorption of the enzyme is the common problem as I mention and solid support material like alumina, silica, porous glass, ceramics and diatomaceous, clay, bentonite and organic material like DEAE-cellulose, starch,

activated car[bon]. So, different type of solid matrix can be use for the immobilization purpose through the absorption techniques.

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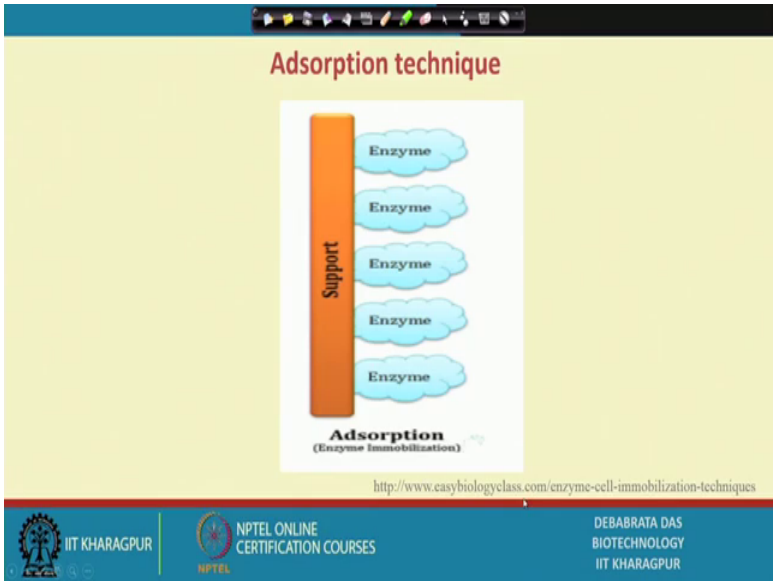

Now, this is how it adhered on the surface of the solid matrix this is the solid matrix and just enzymes simple adhered on the surface of the solid matrix.

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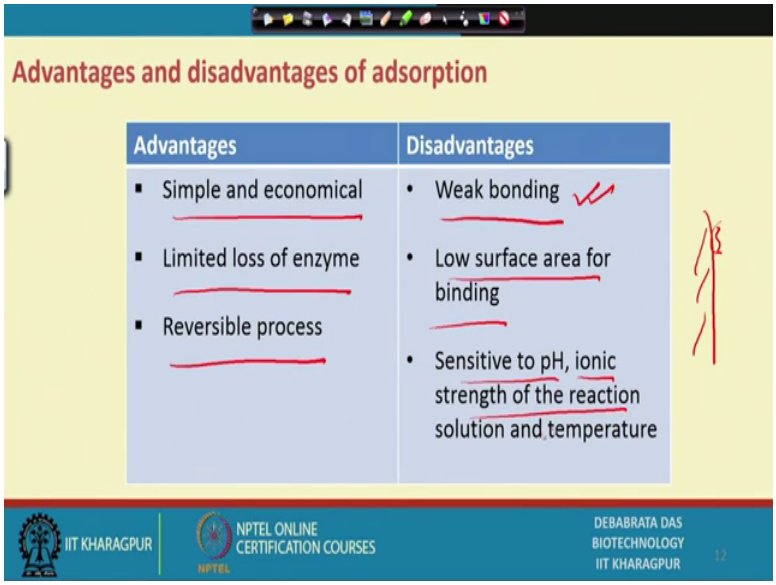

Now, the advantage of this process is the it is very simple and economic, limited loss of enzymes and reversible process and that you know here that here the reversible process with your dissolve the enzyme again you can adhere you can immobilize enzyme on the

solid surface. Now, with the weak bonding this is the major disadvantage. The low surface area for the binding because usually that you know that usually that we use the external surface for the immobilization of the enzymes. So, it depends on the how much surface is available and sensitive to pH ionic strength of the reaction solution and temperature.

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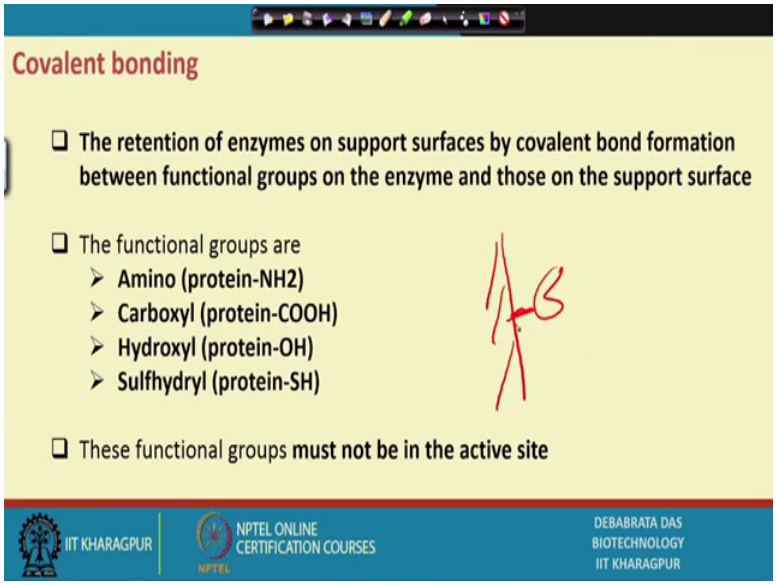

Next is covalent binding. Now, covalent binding is considered as the strongest binding between the suppose, this is this is solid matrix and this is the enzyme. Now, this bonding and how we call is covalent bonding; how the covalent bonding take place? Due to it is the kind of sharing of the electron. So, one electron from the solid matrix one electron from the enzyme they are sharing with each other and then they form the bond and this is very strongest bond. So, you know that, so, retention of the enzymes on the support covalent bond formation between the functional group of the enzyme and those of the support surface. So, this is I can give the example of CMC cellulose carboxymethyl cellulose that is largely use for immobilization purpose.

Now, here that functional group may be amino group, maybe carboxylic acid group, maybe hydroxyl group, maybe sulfhydryl groups this functional group must not be in the active site. So, as I as I this thing as I mention in case of adsorption phenomena that your active site should not take part for the development of covalent binding.

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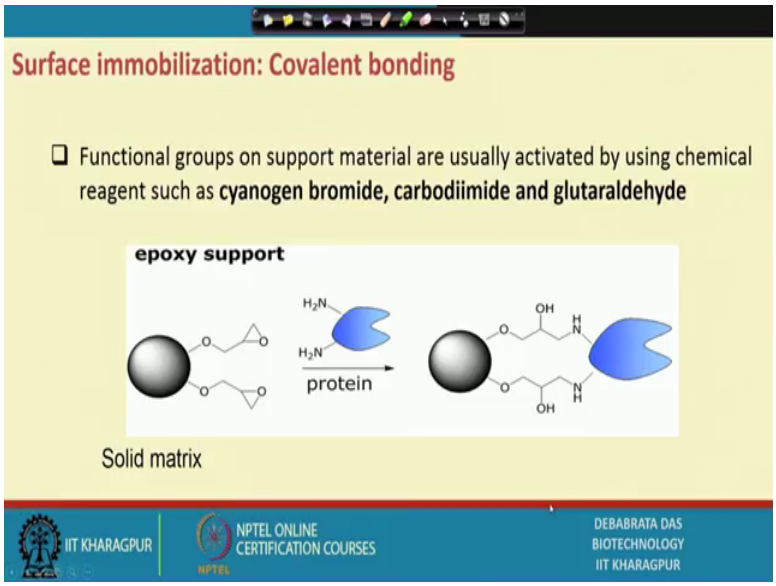

Now, this is the one example that I have given here that I think it will be very cleared this is the solid matrix and this is the protein how protein this is protein nothing, but protein enzyme nothing, but protein molecules and this is how adhered on the surface of the covalent binds with the solid matrix this has been shown here. This is the functional group support material usually activated by using the chemical reagent sizes such as cyanogens, bromide and carbodiiomide and glutaraldehyde. Now, one disadvantage of this process that when we use any kind of covalent bonding we use lot of chemicals and due to use of lot of chemicals there is a every possibility the enzymes will lose its activity. This is the one major drawback we have as per the covalent binding is concerned.

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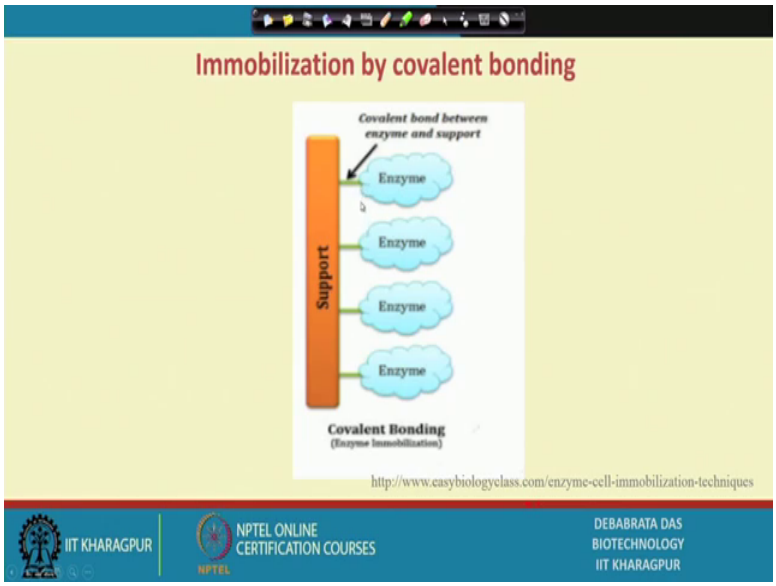

So, this is this is kind of example that this is this is the bond covalent bond I showed you before also.

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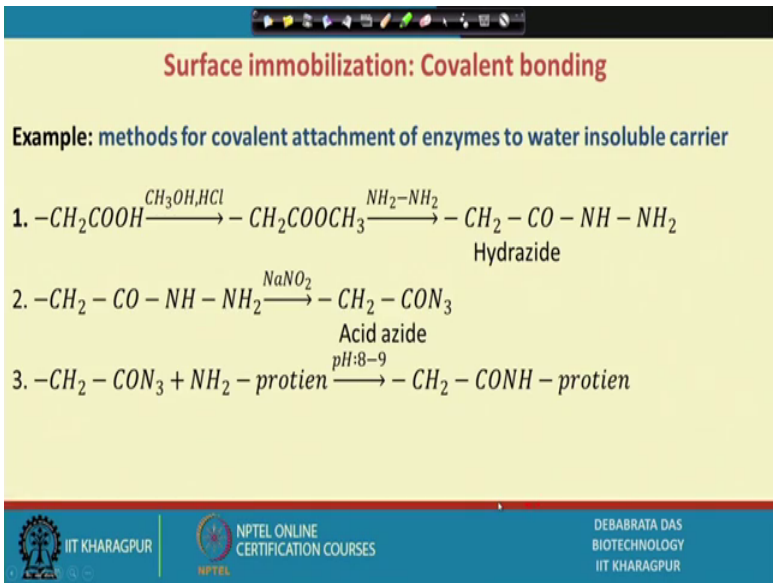

And, this is the chemical reaction this let me explain that one or two, this is the carboxylic acid group, am I right? In presence of this methyl alcohol and this hydrochloric acid, the methyl group will be at is and this is hydrazine, hydrazine this when it reacts then hydrazide formation is there this is called hydrazide. Now, this when undergo the diazo reaction it gone the azo group acid azide this acid azide when it reacts

with the amino group of protein it forms the CO, CONH that into protein. So, this is the how they can covalently bind with the solid matrix. Now, you can see how many steps are there you will state with methyl alcohol HCl then hydrazine, then you have sodium nitrite, then pH you have to maintain 8 to 9 so, since so, many different steps are involved for this covalent binding process.

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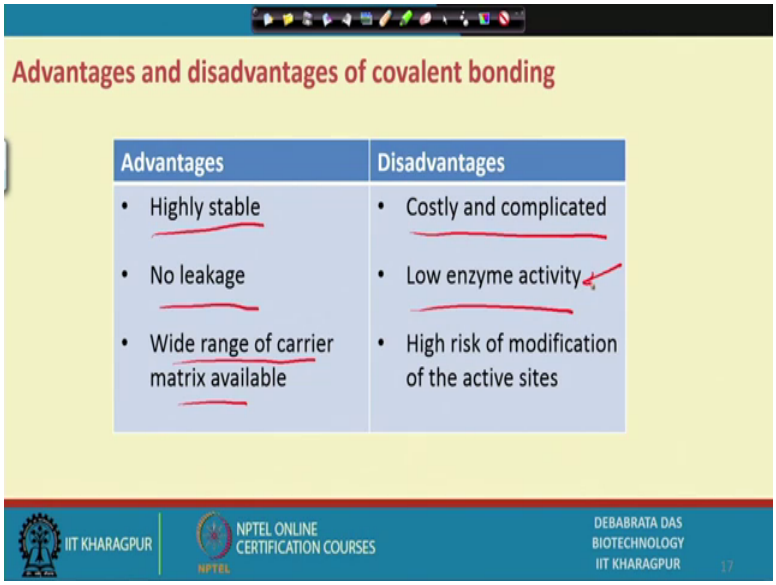

So, what are the advantage and disadvantage? They are highly stable, no leakage and wide range of carrier matrix are available. Now, these processes are very costly and complicated. Low enzyme activity. Why, low enzyme activity? Because during the immobilization the loss of activity of the enzyme will take place and high risk of modification of the active site ; they because since you are you adding lot of chemicals there is a every possibility of the modification of the active sites [Noise].

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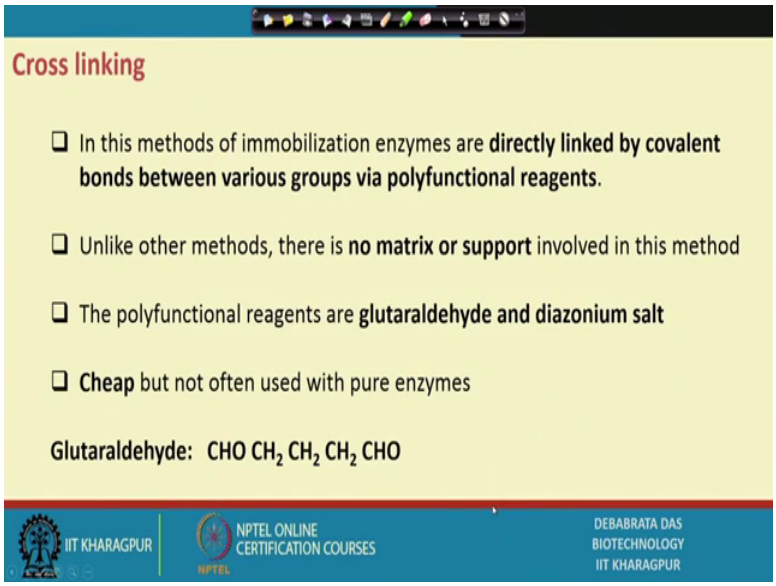

Now, cross linking; another very interesting technique, what do you call cross linking.

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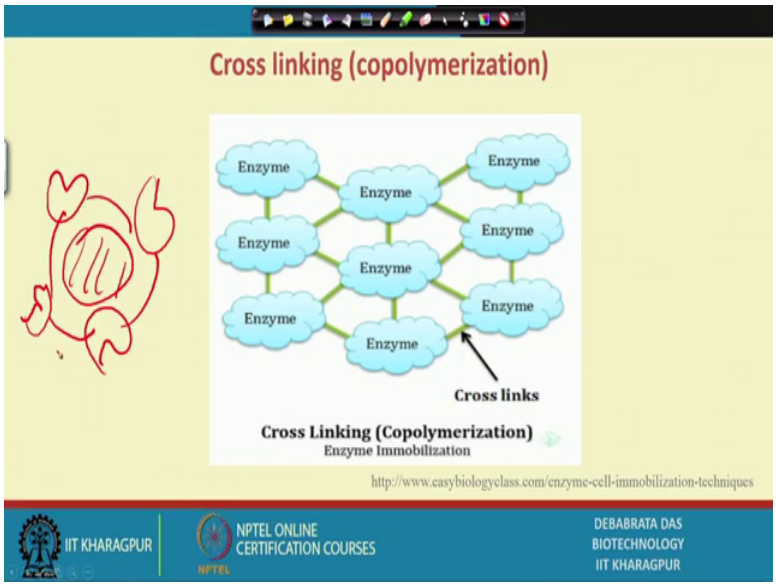

Cross linking means suppose this is this is like this is the enzyme that you know that this is cross linked with enzyme. This is the other enzyme like this you can you can see that how they are cross linking. So, I can I can I can explain like this I think your conception will be little bit clear , that suppose this is the enzyme am I right and this is the another enzyme this is another enzyme, this is another enzyme like this. So, this is like this they cross link with each other.

So, we use some kind of cross linking agent which hold the enzyme together there would this enzyme holding this enzyme and in this enzyme this enzyme holding this enzyme and so, this is called cross link.

Now, with the inside if you put some kind of solid matrix then what will happen, this will be embedded on the surface of the solid matrix. So, this is no interaction with the solid matrix, but you know that enzymes through cross linking they can have this is very strong this is also covalent binding [Vocalized-noise] that you know enzyme to enzyme binding also covalent binding. So, it is also a strong binding, but they are not no interaction with the solid matrix like covalent binding. The cross linking they then the covalent linking between the enzyme and enzyme.

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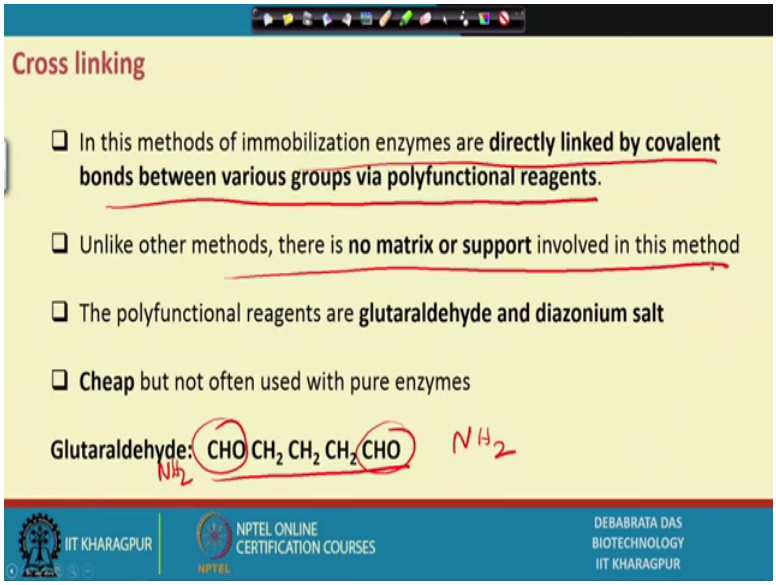

Now, it is let me tell you that this cross linking that in this method that immobilization enzymes are directly linked with covalent bond between the various group of polyfunctional agent and what is the polyfunctional agent, here? This is a glutaraldehyde. Now, what is the formula of glutaraldehyde? CHO, CH 2, CH 2, CH 2, CHO. So, we have two CH2 CHO group, am I right? So, this will bind with this will form compound with amino groups of amino acids here amino group amino acids though there how they are holding the enzyme together.

Now, unlike other methods no matrix are support involved in this method because say it maybe may be done in presence of matrix, it may not be or may be done without the

presence of matrix. The polyfunctional reagents are the glutaraldehyde and diazonium salt and cheap, but not often used for the pure enzymes.

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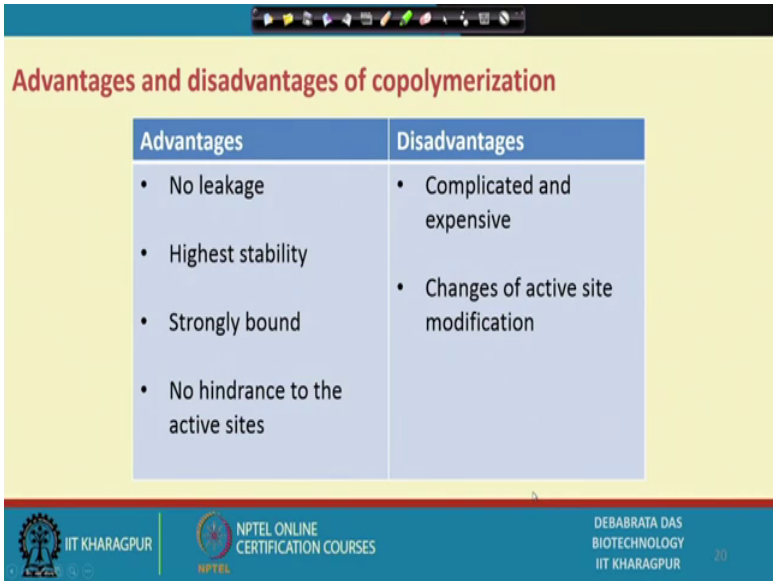

Now, if you look at the advantage and disadvantage of this process, that is no leakage, highest stability, strong bound and no hindrance to the active sites. Now, here disadvantages is a complicated and expensive and changes of active site and modification. So, these are the different problem that we face with the cross linking.

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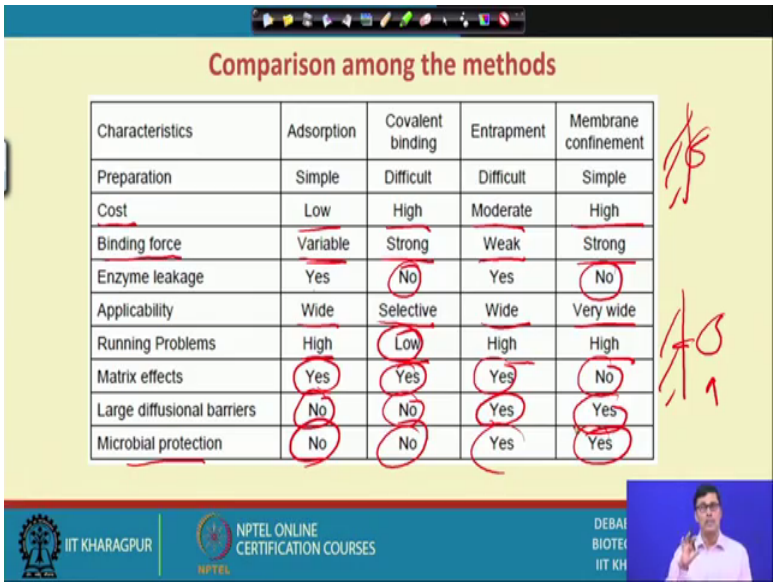

Now, this is a very interesting table. Now, this table they will tell us that how you select the immobilization techniques for the, your particular enzymes. Now, one thing I want to stress here that all immobilization technique is not suitable for all enzymes. So, you have to you have to select the enzymes, we select the mobilization technique on the on the basis of your requirement, I because this table if you look at this that will we have on the basis of certain characteristics, what actually we are looking for.

As for example, suppose their preparation. So, first point is the preparation. What do you mean by preparation? Preparation we mean that that you know that whether the methods of preparation is easy what you are looking for or it should be difficult, if is simple then it is easily. So, if you one of the criteria for immobilize choosing the immobilization technique that the preparation techniques with the simple then we should go for adsorption technique, this is the simple.

But, when you go for covalent or entrapment, it is very difficult or membrane confinement it is will be compatibly simple, but now if you if you look at the cost in case of adsorption it will be low and covalent will be high, entrapment will be moderate and membrane confinement will be high.

So, because if your cost criteria is more then; obviously, you go for adsorption technique because your monetary involvement with less. Now, binding force there will you will you bond the strong binding between the solid matrix and the enzymes? Now, if you want solve strong binding then you should go for covalent binding and membrane confinement, but this is the adsorption binding force would be very weak and this is also this is variable or it will be very weak.

Now, enzyme leakage the what you want that enzyme leakage should be no leakage of the enzyme or you can preferred the leakage of the enzyme is permissible in your system? Now, if you want that there should not be any leakage in your system then you go for covalent technique because covalent and membrane confinement technique otherwise adsorption technique and interpret things there is every possibility of leakage. Applicability; applicability means how widely it is applied. Now, adsorption widely it is applied, then covalent binding is very selective particularly this is used in case of the preparation of some biosensor and this is the entrapment also widely use and membrane confinement been very widely used.

Running problem during the operation of the process that what is the characteristics of that immobilization techniques? that that is the running problem will be high, low, high or high. If you want that running problem should be as low as possible we should go for covalent binding techniques. Now, matrix effect that that whether this process is going to affect the matrix that here is the yes, here is the yes in covalent binding is yes entrapment yes, but in case of membrane confinement no.

So, now large diffusion barrier; now, in case of adsorption no diffusion and barrier covalent binding, low diffusion barrier because your enzymes is there and it is outside. So, you substrate can easily interact with the enzyme, but in case of entrapment we have jump diffusion barrier and membrane entrapment they inside. So, you know there will be every possibility that less diffusion of the substrate or let diffusion of out of the product that will take place.

Microbial protection if you look at now in case of adsorption no microbial protection because in case of adsorption the outside the solid matrix the enzymes are immobilize, there is no protection. In case of covalent also no protection, but entrapment since it is inside there is a protection for microbial attack and here also membrane confinement since it is inside the membrane there will be some kind of protection for the microbial attack. So, you know it depends on the, what you are looking for on the basis of your requirement you should choose the technique.

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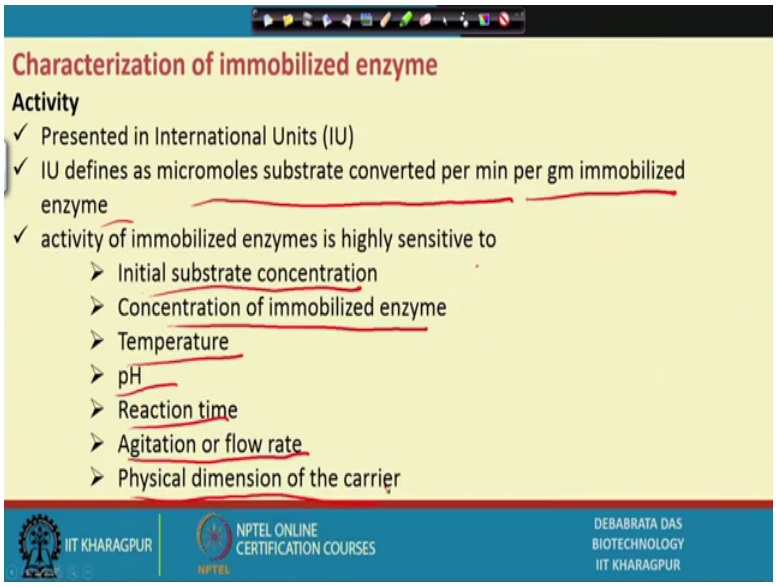

Now, next point that is important, how you characterize the immobilized enzyme? Now, because (Refer Time: 28:47) we have it usually presented by the international unit and international unit is defined as that micromoles of substrate converted per minute per gram of immobilized enzyme . Now, activity of the immobilized enzyme is highly sensitive to initial substrate concentration, concentration of immobilized enzyme, temperature, pH, reaction time, agitation or flow rate and physical dimension.

One thing I, I mentioned in the last class also that that in case of immobilize system suppose when you use the soluble enzyme when we immobilized we immobilized on a solid matrix, so, naturally it will converted into solid heterogeneous mixture and since it is heterogeneous mixture solid liquid be mixture. So, when you put it in a liquid solution there is every possibility the solid will settle down at the bottom. So, there you should required some kind of agitation to the substrate can freely interact with the enzyme and keep the product.

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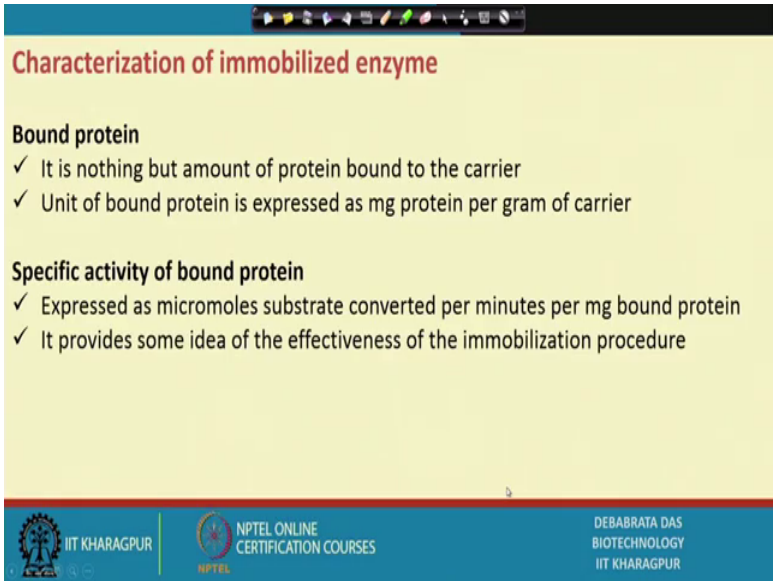

Now, bound enzyme; this is bound enzymes is very simple that how much of protein is bound on the solid matrix. This is usually expressed as milligram of this is usually expressed as milligram of protein per gram of solid matrix. Now, specific activity of the bound enzyme protein can be expressed as micromole of substrate converted per minute per milligram of bound protein. This it provide some idea the effectiveness of

immobilization procedure. So, you know that the, this is how we can find out the activity of the immobilized enzyme.

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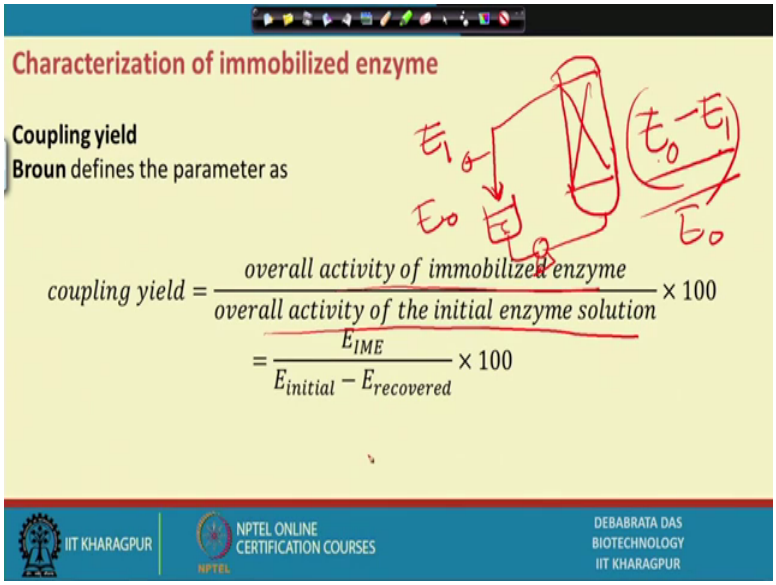

Now, finally, I want to tell you that immobilize system there is a one thing that is called coupling yield or you know coupling efficiency. What do you mean by coupling efficiency? The overall here you see the overall activity of the immobilized enzyme and overall activity of the initial enzyme solution present. So, what I showed you suppose, that this is this is the solid matrix am I right now you this here you put the solid matrix here you have enzyme solution. So, you know initially you know the enzyme activity, now with the help of pump, you pass your solution like this and you recycle back like this.

So, a time will come your column will be totally saturated with enzyme. Then this activity will be constant let us assume this is the activities E 1. So, how much enzyme is absorbed in the system E 1, E 0 minus E 1 am I right? So, this is like this the overall activity immobilize. So, how much enzyme immobilize E 0 minus E 1, how much initially it is present, E 0. So, that ratio is called coupling yield. So, this is how we can easily find out.

So, in this particular lecture I tried to discuss different immobilization techniques and there are different like you know we have with the immobilization technique can be divided into two type different types of immobilization techniques we have come across

and this selection is on the basis of the what kind of enzyme you are handling, the soluble enzyme or insoluble enzyme. So, on the basis of that we have given the classification of the immobilization technique. Now, what we have discuss that what do you mean by adsorption technique, what do you mean by covalent immobilization technique, what do you mean by cross linking technique and what do you mean by meantime membrane entrapment and hollow fiber entrapment all this technique we tried to discuss.

Now, question come when you come to any kind of immobilization which technique will be suitable for a particular enzyme, then you have to first you have to find out the criteria; what you are looking for, is the is the preparation your binding force should be strong or you can reuse the enzyme again and again, you want the microbial protection of the enzyme. So, on the basis of that you have you can select what enzyme immobilization techniques you should use and finally, I tried to discuss the how you can express that the activity of the immobilized enzyme both with respect to the solid matrix, with respect to the bound protein and also I try I explain what do you mean by coupling efficiency of the immobilization system.

Thank you very much.