Course on Industrial Biotechnology
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Lecture 14
Immobilization Techniques.

I welcome you back in the industrial biotechnology course.

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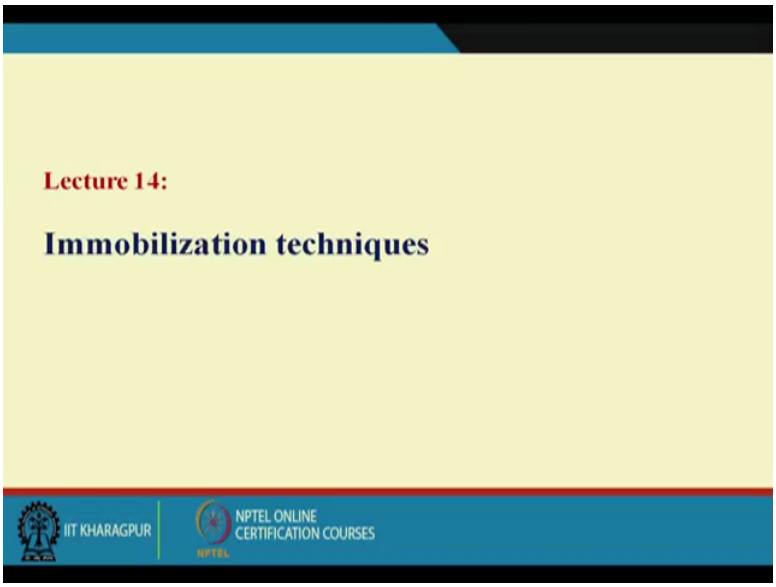

Today I am going to discuss immobilization technique immobilization technique has lot of application in the industry both for the immobilization of the enzymes as well as immobilization of the whole cells now initially I will discuss the immobilization of enzyme and then in next I'll discuss the immobilization of the whole cells now if you look at the definition of what you mean by immobilization?

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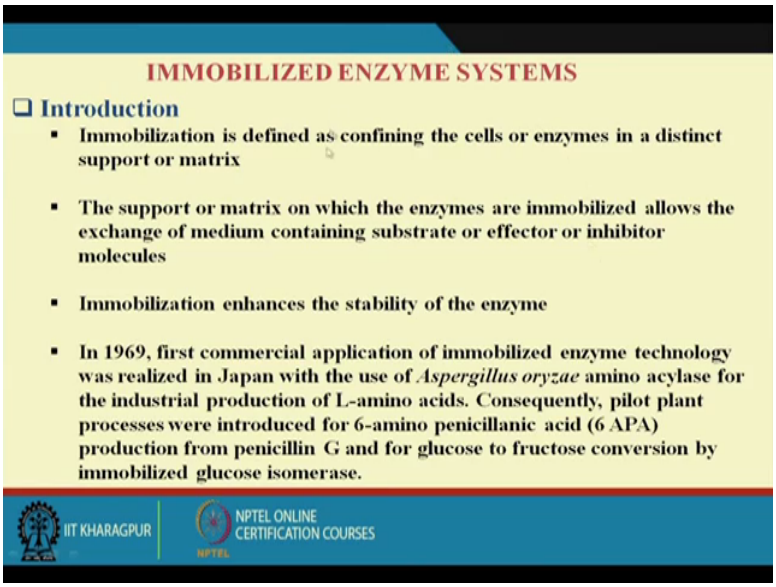

Immobilization is defined as confining the cells or enzymes in a distinct support or matrix just is immobilized suppose this is a solid matrix and it immobilised on the surface of the solid matrix so that it doesn't move because your immobilization means the mobility of the enzyme or mobility of the cell will be arrested.

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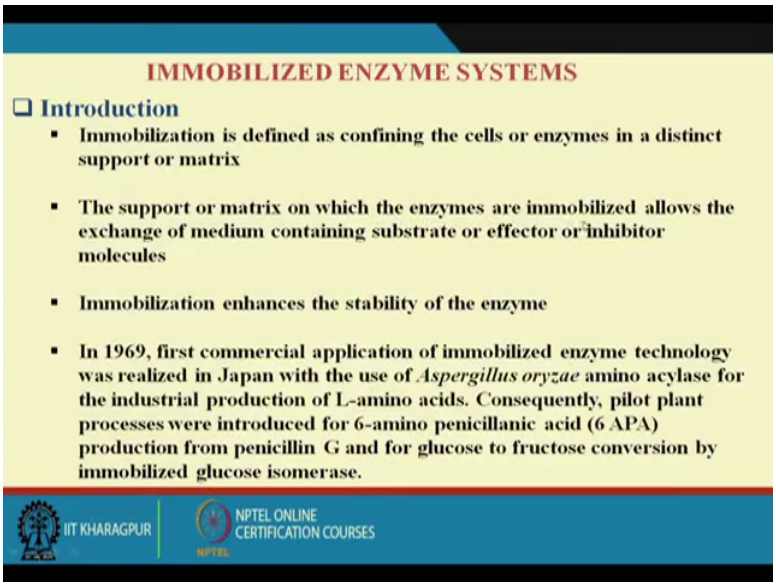

And then the support Matrix on which the support matrix on which the enzymes are immobilized allows the exchange of media containing the substrate or effect or or inhibitor molecules. The immobilization enhances the stability this is another advantage we have by immobilization we can increase the stability of the enzyme to a great extent.

In 1969 first commercial application of immobilized enzyme technology was realized in Japan with the use of Aspergillus oryzae amino acylase for industrial production of L amino acids. Consequently pilot plant processes when introduced for 6 amino penicillanic acid from penicillin G.

Because this is largely used by the industry also for glucose to fructose conversion by immobilized glucose isomerism enzyme mainly this is for the production of high fructose corn syrup because in the western country most of the confectionary they use the high fructose corn syrup as the sweetening agent.

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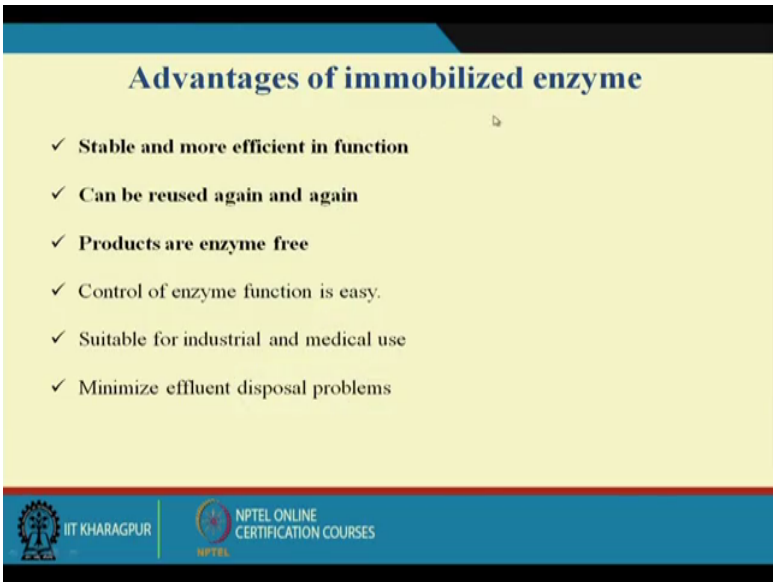

Now the advantages of immobilized enzyme is that a stable and more efficient in function can be reused again and again this is the very advantage very important because when we talk about the free enzyme or free cell after the reaction is over the enzymes or the cells impurity in the reaction mixture so we have to remove that.

But in case of immobilized enzyme or immobilized cell when you after getting the product product is free from because solid mass you can easily separate from the souble material the soluble material will free from the enzymes as well as immobilized enzyme as well as immobilized cell so it is free from the enzymes and cell so purification process will be simpler.

So not only that when you immobilized the enzyme or immobilized cell you can reuse the cell or enzymes again and again that is the major advantage of the immobilized enzyme process or immobilized whole cell system.

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Now products are free from enzyme free or cell free and control of enzyme function is easy because and suitable for industrial and medicinal use particularly in the medicine they use I can tell you due to the genetic disorder sometimes some of us unable to secret the essential enzyme so which in that case we will have to give take the essential enzyme everyday with our drug.

Now if you if we immobilize the enzyme in a solid metric and put it in the glass stream so we don't have to take the enzyme again and again so your blood can use that enzyme again and again until and unless that deactivation of the enzyme take place.

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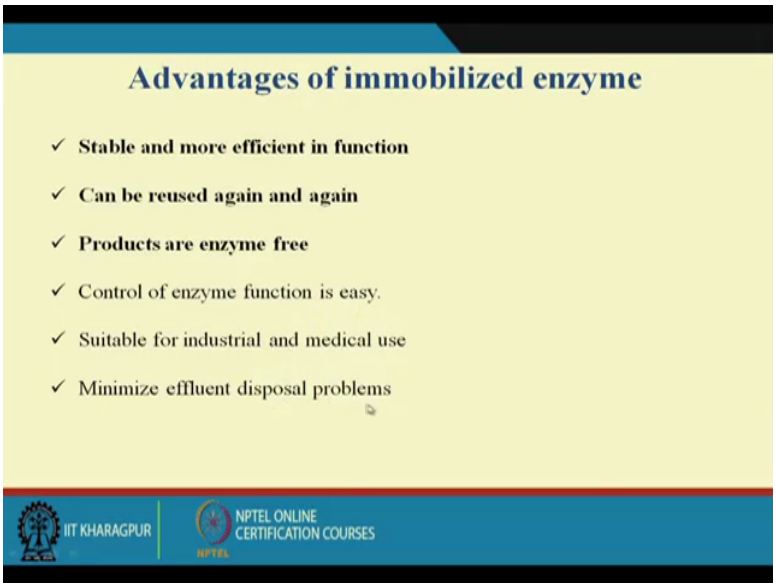

Now minimize the effluent disposal problem this is because since it is free from enzyme and cell so effluent disposal problem will be little bit simpler.

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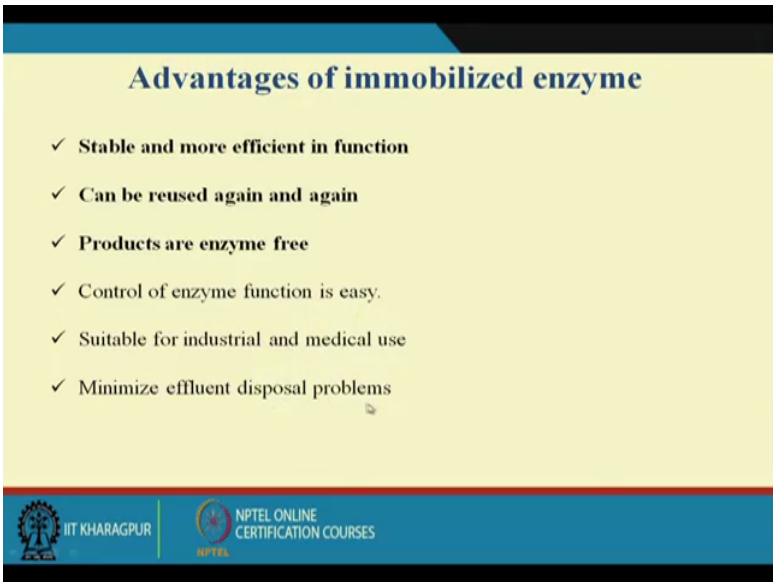

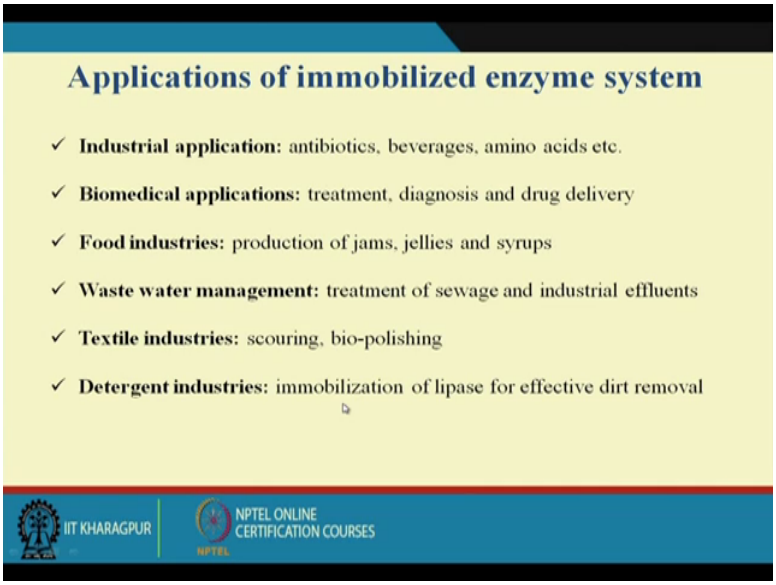

Now application of immobilized enzyme industrial application antibiotic production just I told 6 amino pallicilinic acid from penicillin, beverages industries because I can I can give the simple example of beer making industries. One important problem with the beer industry is the chill problem chill proof beer.

Chill proof beer means beer use any surf under chill condition and as you know that among all the alcoholic beverages the beer is is considered as a energetic drink because why because it contains good amount of protein. Now protein as we know at low temperature and high temperature is precipitated out so since we serve beer on the chill condition.

And if you are liquid contents that you know beer content bigger protein molecule there is a possibility that there should be precipitated out. So it will get the haziness in the beer which is undesirable so what you do basically we have immobilized whole cells (())(5:58) we immobilize the photolytic enzyme and pass the beer through this.

So that (())(6:03) in the higher protein molecule that will be degraded in the smaller protein molecule and which will not be precipitated in hand low temperature this is how it is used in the beverage industries.

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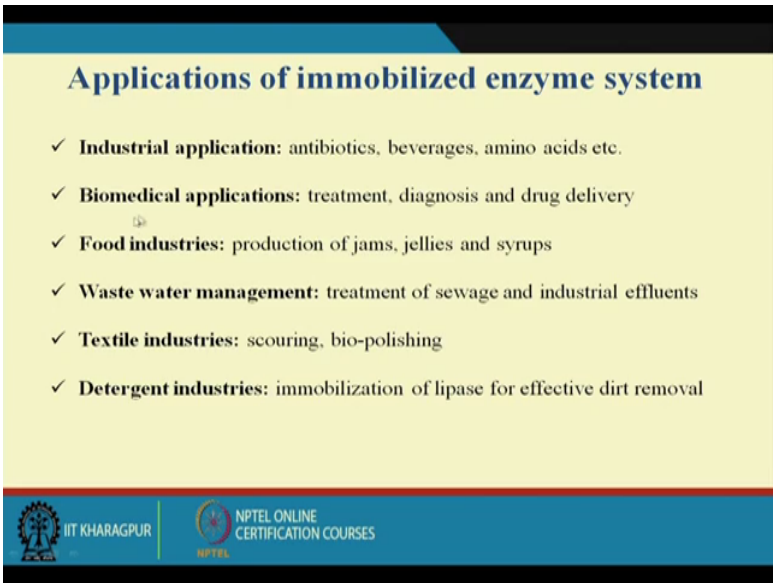

Also amino acid production biomedical applications just I told you what the treatment diagnosis and the drug delivery system. Now here I want to diag diagnosis system let me tell you that particularly when we talk about the enzyme sensor that enzymes are very specific to the substrate now suppose during the Olympic games sort during the any other games the athletic they are not suppose to take any kind of stimulant.

And alcohol is the one of the stimulant so suppose we have we have the alcohol bio sensor then if we take the blood sample from the athletic and just insert this prob of the enzyme sensor that alcohol is inside if that person that athletic its take any alcohol then it will immediately detected whether the athletic taken any alcohol or not. So immediately we can like you know like we can find out the detection of different stimulant that is present in the we can easily do that

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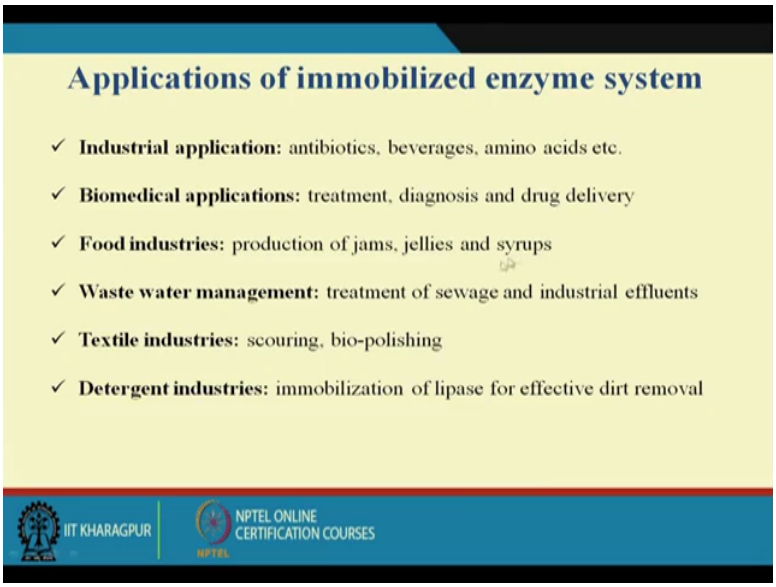

This is the diagnosis purpose and then food industry we have production of jam, jelly and syrup that is used then waste water treatment process particularly sewage and industrial effluents I I I I want to take the example of of this tricking filter trickling. What is the trickling filter? Basically it is the gravels on which the cells are immobilize on the surface then we sprinkle the waste water from the top/

When we trickle down then it comes in contact with the (())(7:58) and degraded and your waste water will be stabilize this is how the.

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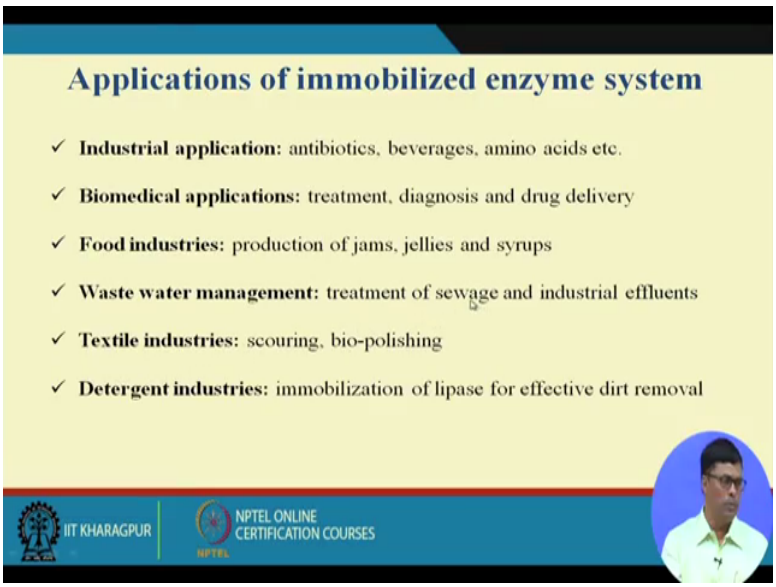

Treatment of sewage water or industrial enzyme that take place. The textile industry is use for scouring and bio polishing then detergent industry immobilization of lipase for effective dirt removal because the lipase not will lipase we can use the proteins also that can remove the blood strength that preserve in the in the (())(8:27)

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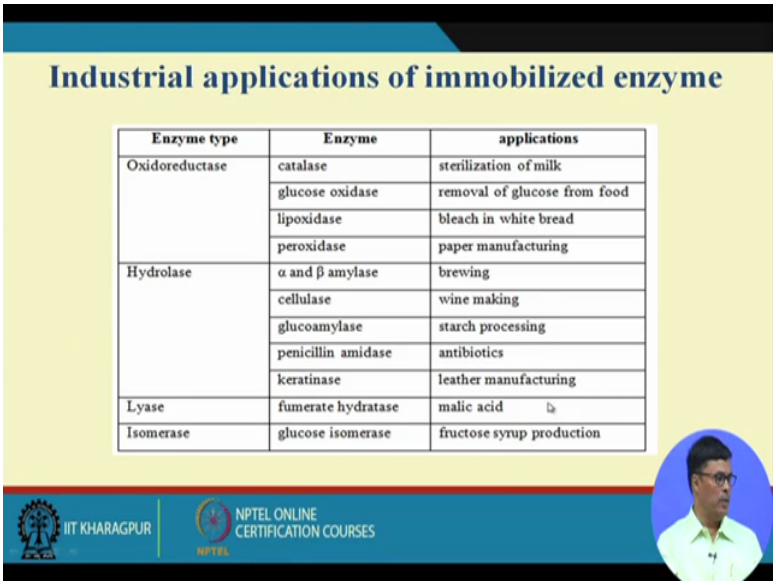

Enzyme type	Enzyme	applications
Oxidoreductase	catalase	sterilization of milk
	glucose oxidase	removal of glucose from food
	lipoxidase	bleach in white bread
	peroxidase	paper manufacturing
Hydrolase	α and β amylase	brewing
	cellulase	wine making
	glucoamylase	starch processing
	penicillin amidase	antibiotics
	keratinase	leather manufacturing
Lyase	fumerate hydratase	malic acid
Isomerase	glucose isomerase	fructose syrup production

There or in the detergent industy that largely used then the industrial application of immobilized enzyme we have oxidoreductase some examples we have given this we have the example are catalase or glucose oxidase, lipoxidase and peroxidise now this are catalase use for the sterilization fo milk, glucose oxidase for the removal of glucose from food and lipoxidase the bleach in white bread.

And peroxidise for the paper manufacturing industries. Similarly hydrolase we have alpha beta amylase use in brewing industries, cellulase in wine making, glucoamylase for starch processing, penicillin amidase is antibiotics, keratinase in leather manufacturing. Lyase who use for malic acid production and isomerise is used for fructose syrup production.

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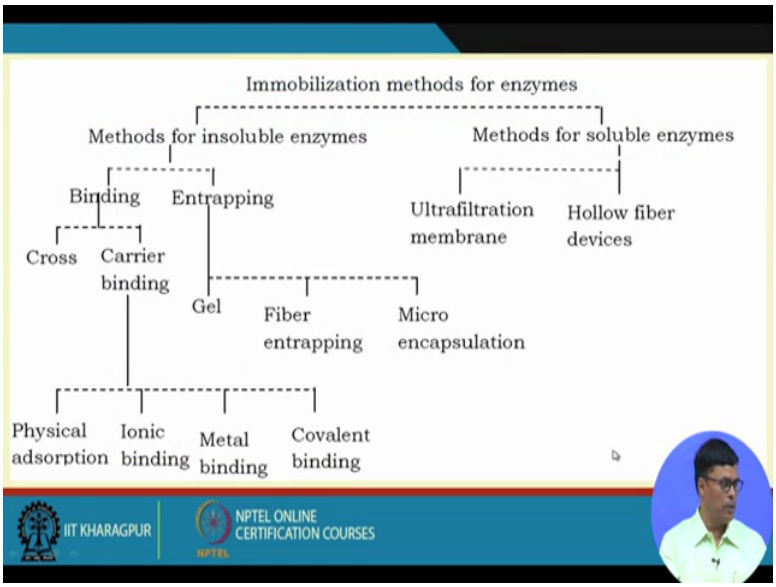

Now let me tell you the immobilization techniques now immobilization techniques broadly divided for 2 different type of enzyme 1 we call soluble enzymes and another we call insoluble enzyme.

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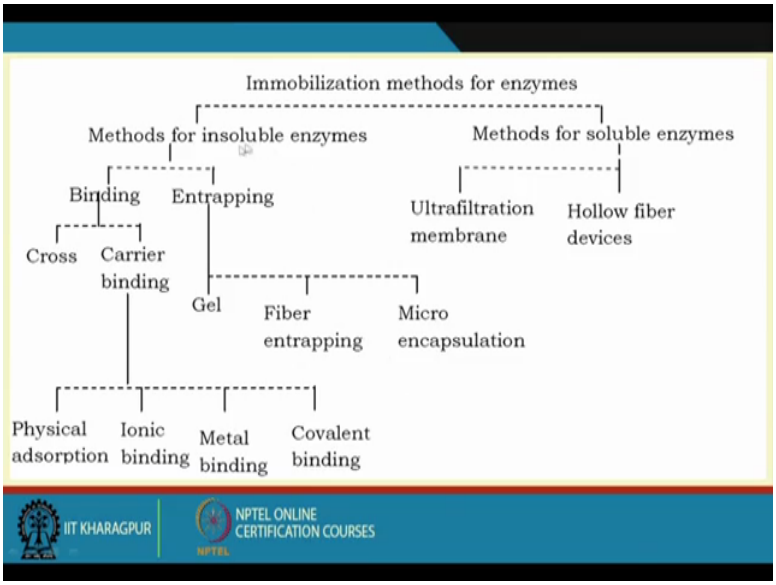

So we have methods for the insoluble enzymes we have methods for the soluble enzymes now method for the insoluble enzyme we have it can be differ into 2 types 1 is binding another is entrapping. Binding we have cross linking and carrier binding again carrier binding we have physical absorption, ionic binding, metal binding and covalent binding. Entrapping we have 3 types 1 is gel entrapment, fiber entrapping and micro encapsulation.

And in case of soluble enzymes we have 2 types 1 is called ultra filtration membrane another is hollow fiber devices. So these are the different immobilization technique that is used for the immobilization of the enzymes. And finally I can discuss how we select the immobilization technique for the immobilization of the enzyme at the end of my lecture I shall show you.

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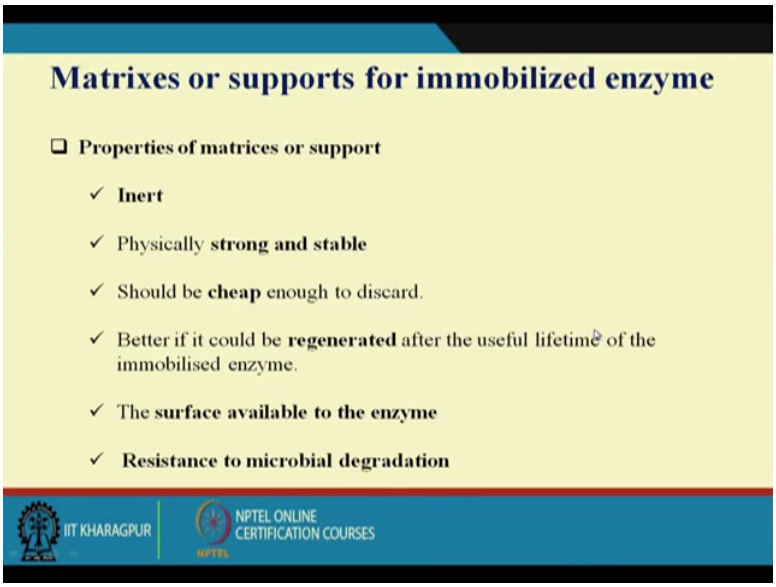

Now the important thing that is we have in the immobilization of the enzyme this is the solid matrix the property of the solid matrices play very important role and what should be the property of the solid matrices it should be inert and physically strong and stable. Because suppose I can give the example suppose the immobilize the enzyme on a solid matrices and then we put a mechanical stirrer.

And if it is weak then what will happen the your solid matrices will be disintegrated so it should be strong it should be strong enough to withstand the CR forces so then it should be cheap enough to discard. Because if it is very costly I can give a typical example here again it depends on the type of solid matrices here used.

I can give the example of biosensor where we use the platinum foil and as you know platinum is more costlier than gold. so now one advantage with the platinum foil is that we can reuse the solid matrices again and again once we use that again when when your activity of the enzyme decreases then you take it out/

And and put it in the oven so that all the organic material we will bound of again you can use this platinum foil for the immobilization purpose. So you can number of use of (())(12:03) point to be very very high. Now if you use in that case if you use the polymeric material though it is very cheap but we can reduce again and again that is the major problem that we have.

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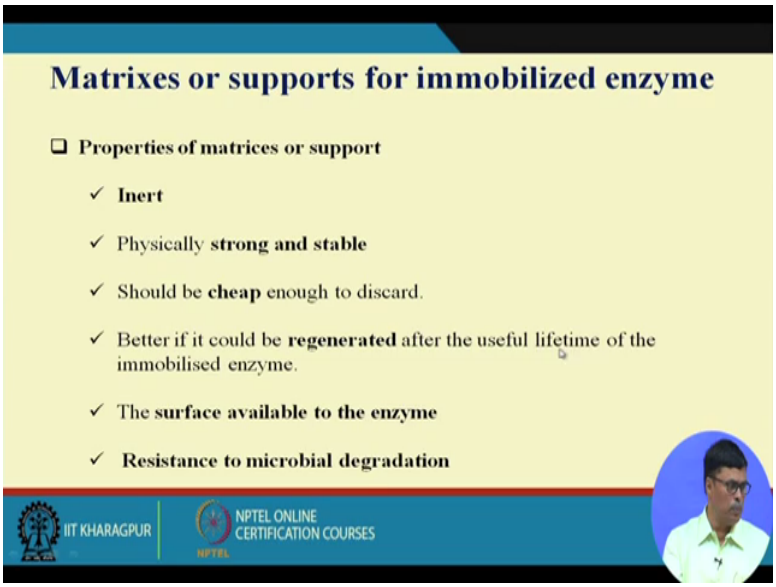

Now better if we could regenerate after the useful life time of the immobilization. the regeneration is a very important characteristics of the solid matrices then surface availability of the enzyme because more surface area more enzyme will be immobilized on the surface. so that is the desirable characteristics of the solid matrices and last one that it is the that it should have a resistance power for microbial at microbial degradation.

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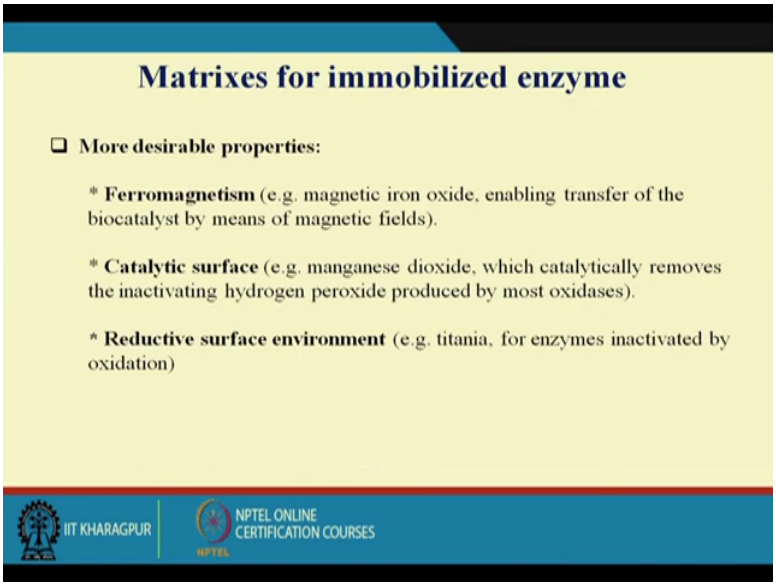

Now matrices for immobilized enzyme more desirable properties that fellow magnetism the magnetic iron oxide enabling the transfer of bio catalyst by means of magnetic field then because I don't have some kind of magnetic field you know that and catalytic so catalytic surface that manganese dioxide when catalytically removed the inactiving the hydrogen peroxide produced by most oxidases enzyme.

So if hydrogen peroxide is produced it has strong oxidising agent. now this is to be removed otherwise it will deactivate the enzymes, and reduction of surface environment that is titania titania the enzymes inactivated by oxidation.

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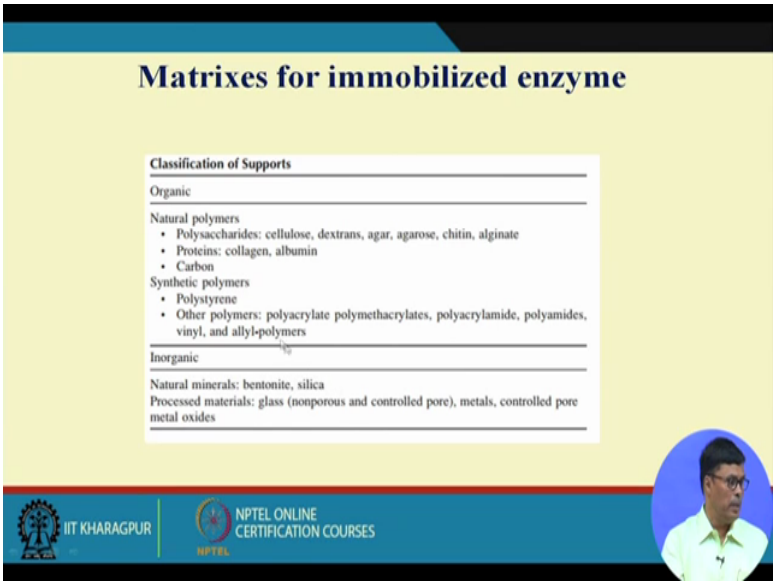

Now there are several solid matrix that is usually used. The ()((13:46)) divided into two types one is organic another is inorganic. Organic we have natural polymers like polysaccharides we have cellulose, dextrans, agar, agarose, chitrin and alginate. Then protein we have collagen, albumin and we have carbon.

Some kind of activated carbons we have used. Synthetic polymer we have polystyrene, other polymers polyacrylate, polyacrylamide, polyamides. In inorganic we have bentonite, we have silica and processed material like glass. Glass we have two type one is controlled pore and the nonporous, we have metal, we have controlled poremetal oxides.

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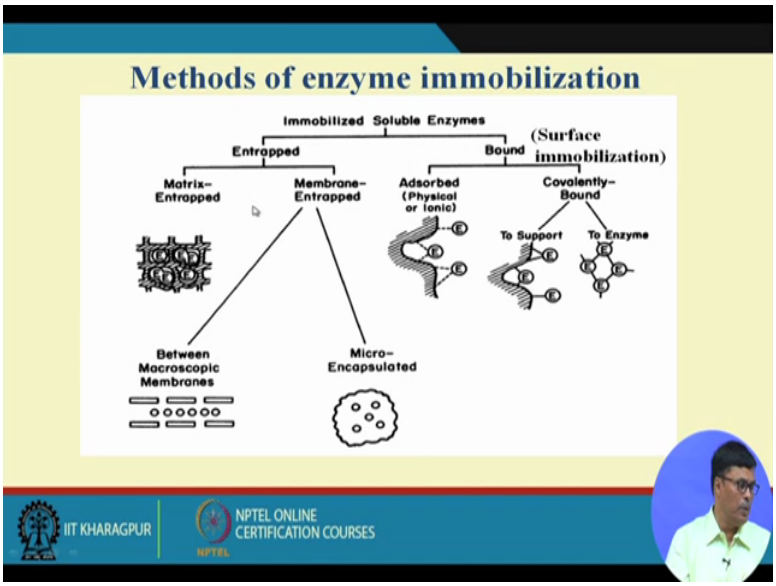

Now methods of immobilization that we have talked about the entrapped. In entrapped basically it entrapped basically inside may be inside the membrane, inside the ()((14:50)) that inside the capsule because that encapsulation, microencapsulation. So I want to show you little bit that suppose in case of gel membrane ()((15:03)).

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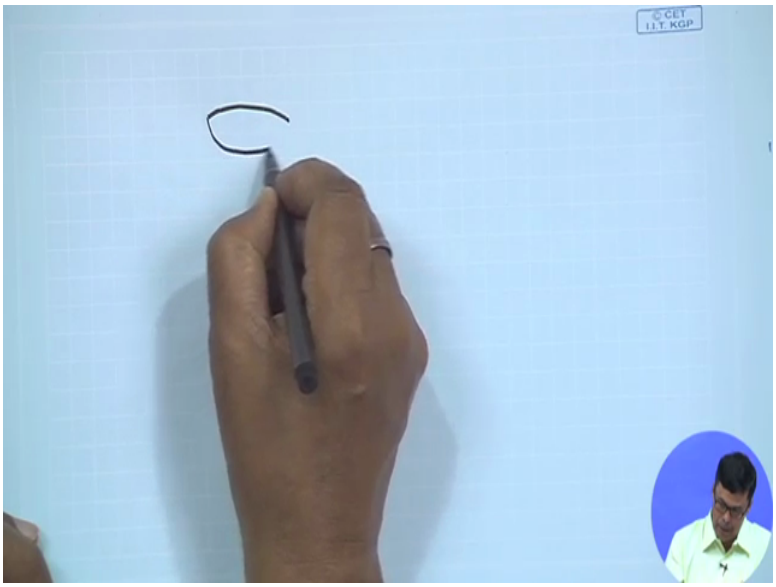

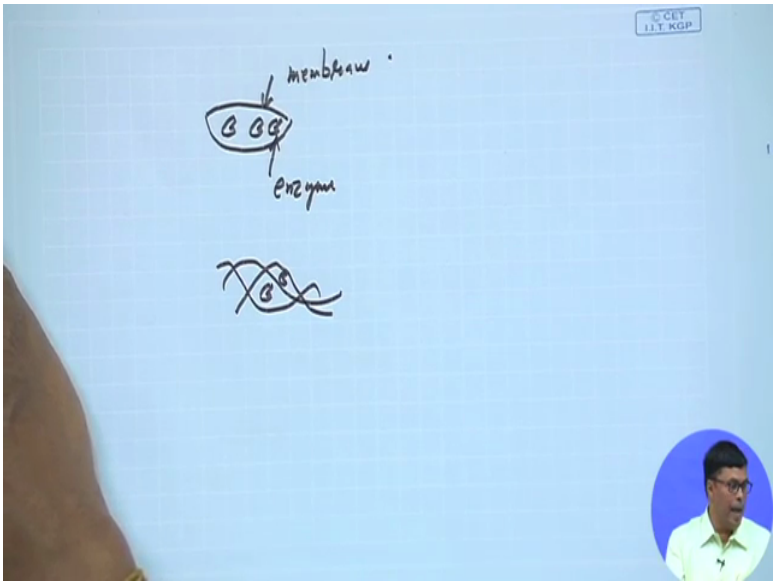

Suppose we have membrane means suppose this is a collagen membrane and an inside inside the membrane we have the enzyme this is enzyme. This is enzyme and this is membrane. now this is the membrane entrapped and we have we have shown you that it can it can interrupt in in the fibre Entrapped also there we have fiber like this.

The fiber it looks like this. So inside the fiber we can have that your enzymes entrapped that also can be done this is the fiber.

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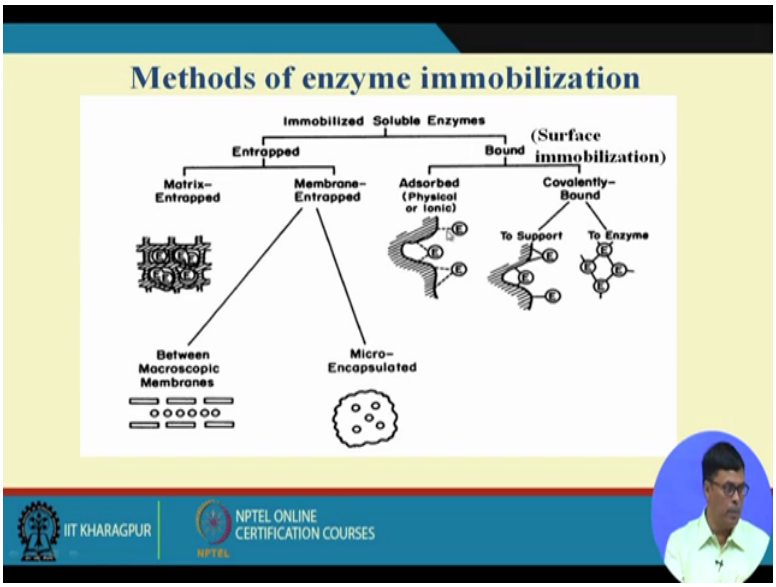

Then absorbed is the most simplest technique. This added on the surface of the solid Matrix but this this addition is due to the bundle all type of forces and as you know that we know that ()((16:03)) type of force is very weak force. Now when you pass your liquid little bit higher flow rate. it has some kind of sharing effects.

And then we see sharing effect. It is possible that every possibility that it may it may detach from the solid particlels go to the strip that is the major disadvantage of the absorption technique. Otherwise this is this is considered as the most easiest technique that is used by the industry.

Now covalently binding covalently binding is largely due to electron sharing. This bond is in between the enzymes and the solid matrix is due to electron sharing. As I show you how it is done. Now this is appears to be the strongest Bond and as I told you that absorption is considered as weakest Bond and covalent is considered as the strongest and is very difficult to detach this enzymes from the solid matrix once it formed the covalent binding with the solid matrix.

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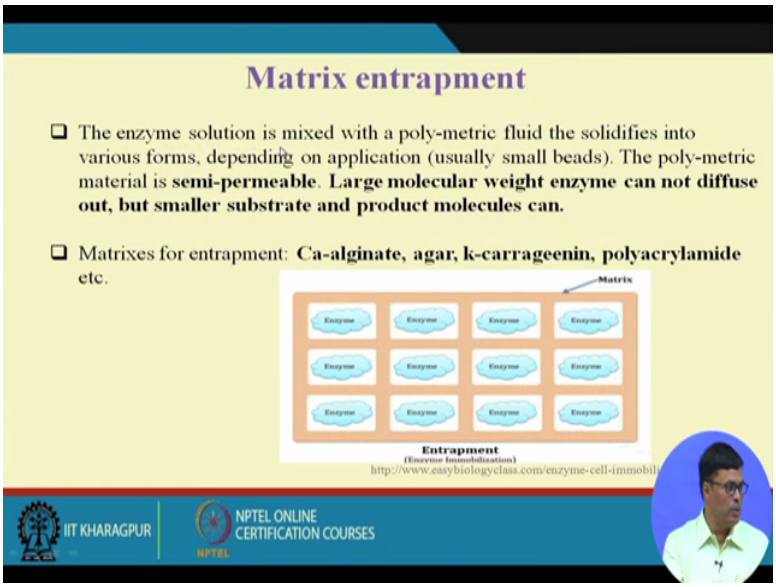

Now matrix entrapment. Suppose this is the enzyme solution is mixed with the poly metric fluid the solidifies that solidifies into various form depending on application. The polymetric material is semi permeable molecular weight enzyme cannot be diffuse out but smaller substrate and product molecules can do can do.

So as for example suppose this is the gel or matrix and you said the matrix the enzymes are immobilized but here your substrate can interrupt through the pole and product can get out through the pole butenzymes can not go. Why? Because enzymes are bigger in size and solid metrics are different times one is ca alginate, agar, k carrageenin and polyacrylamide etc.

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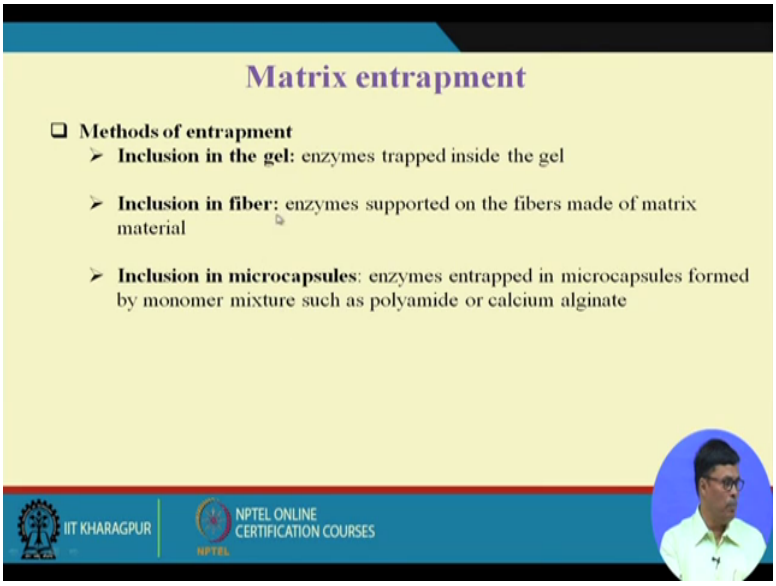

Now that matrix entrapment we have inclusion of Gel. Enzymes are entrap inside the gel because gel it just started to be inside the structure. The enzymes are entrapped inclusion in fibre just I showed you haw it influence inside the fiber. At the micro microcapsulation we know we have seen in day today live we take lot of Capsule for medicinal care.

So like this we have some kind of coating outside inside that enzyme I have given the example of collision membrane that we put inside the membrane. That is also kind of capsulation. Enzyme entrap in the microcapsule formed by monomer mixing ()((18:54)). Particularly this is largly used in the lab that calcium algenide.

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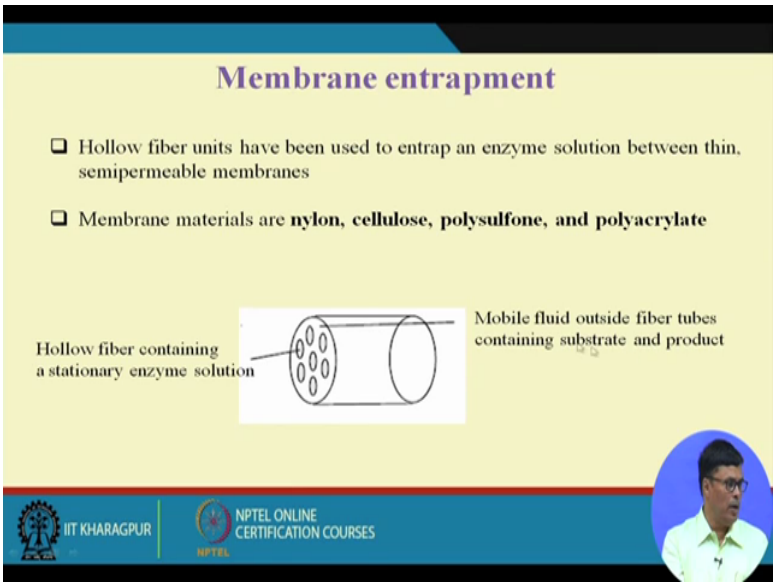

Now we have membrane entrapment. In membrane entrapment we have hollow fiber units has been used to entrap an enzyme solution between thin and permeable semi permeable membrane. Semis permeable are not permeable for all the molecules. It allows only the substrate and product molecule to go in and go up and comes out.

And enzyme cannot go out the we can use the membrane material like nylon, cellulose, polysulfone and polyacralyte. that we use hollow hollow fibre looks like this. This is this is Hollow inside this that enzymes entrapped.

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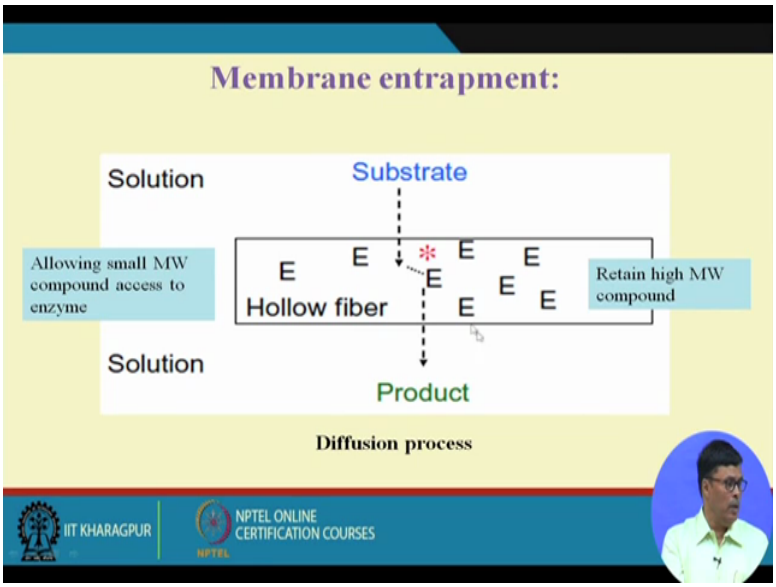

Now membrane entrapment this is solution upside and the inside we have hollow fiber. Hollow fiber inside this the enzymes entrapped. You can see that and your substrate goes in and products comes out. This is kind of diffusion process that you have.

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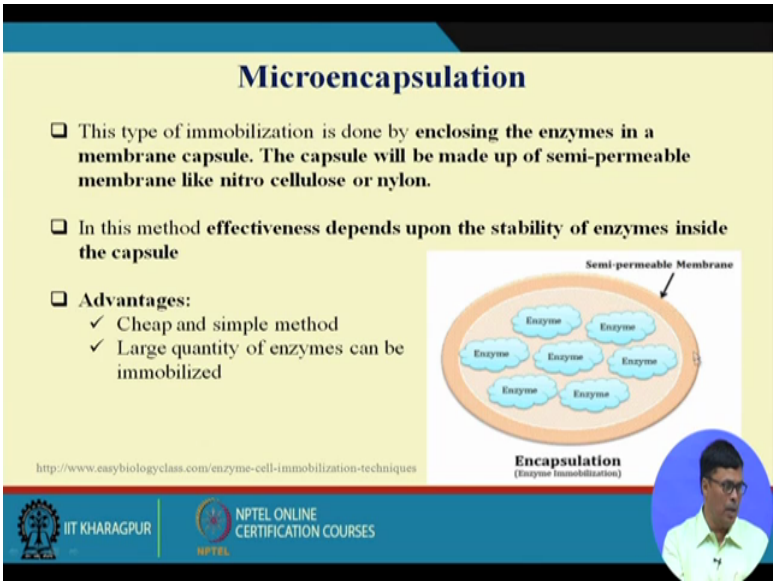

()((20:04)) encapsulation I told you that it is put in the encapsulation clip like this. this type of immobilization is done by increasing the enzyme in a membrane capsule. The capsule is made up of semi permeable membrane like nitro cellulose or nylone. In in this method the effectiveness depends upon the stability of enzymes inside the capsule. Advantage is that this is cheap and simple method and large quantity of enzymes can be immobilized.

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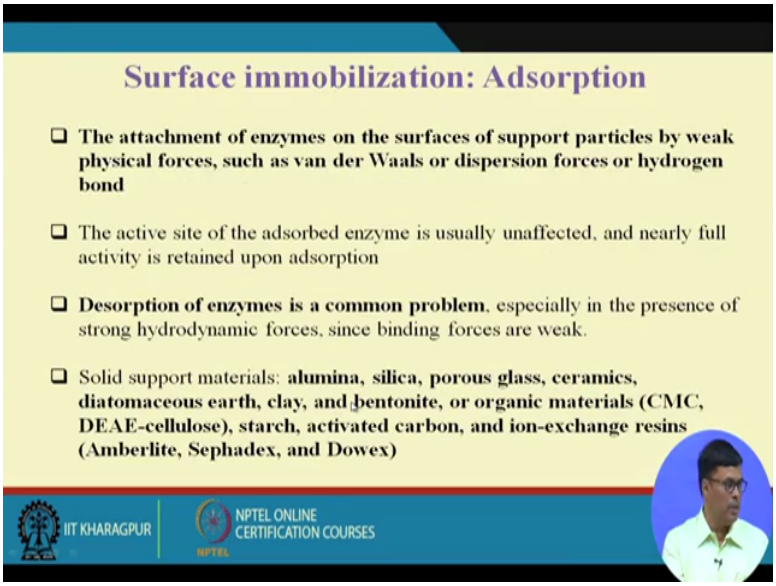

The surface immobilization that is adsorption I told you is the simplest technique nad cost of this technique is very less. But only the problem is that it cannot ()((20:51)) high force. The attachment of enzyme on the surface of the support particles by weak physical force it is van der waals or dispersion force or hydrogen bond.

The active site of adsorbed enzyme is usually unaffected and nearly full activity is retained upon adsorption. Now during adsorption I want to point out one thing here.

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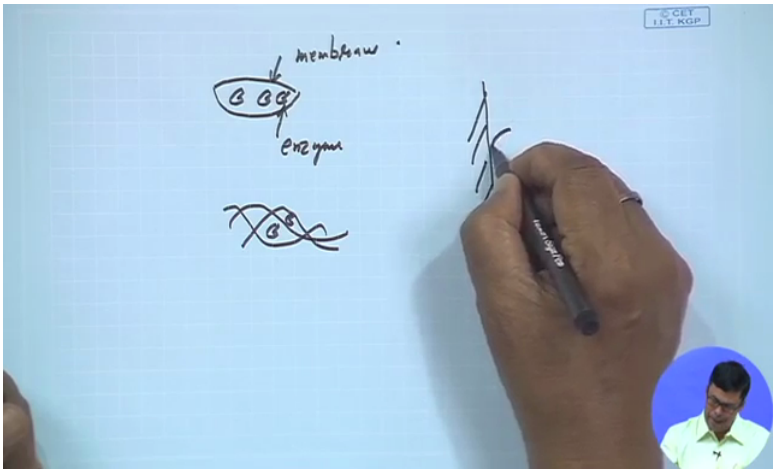

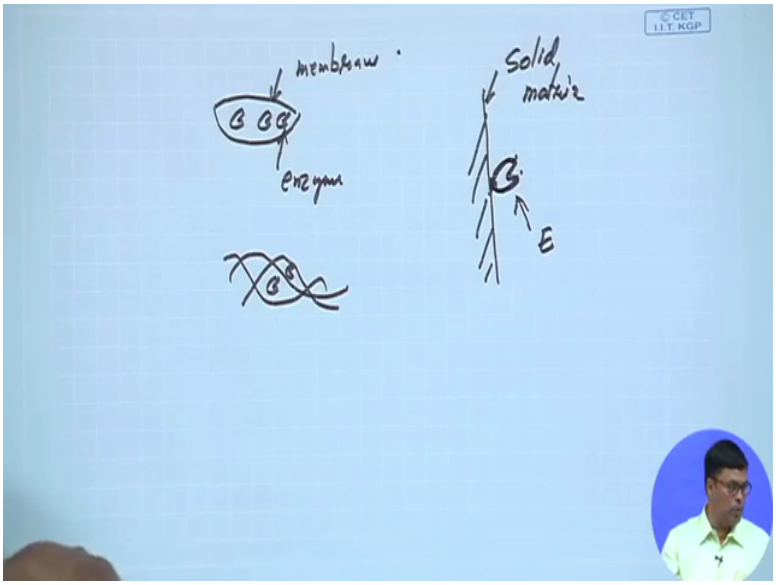

You see suppose this is a solid matrix and this is the enzyme. So when we do any kind of immobilization this is the solid matrix solid matrix and this is the enzyme. now when you do this immobilization we take into account that inactive side, this is the inactive side of the enzyme and this is the active side of the enzyme.

Then we take into account immobilization should be done between the solid matrix and the inactive portion of the enzyme. Not active portion. If we immobilize the active portion of the enzyme then activity of the enzyme will be reduced to a great extent. So this is to be taken into account.

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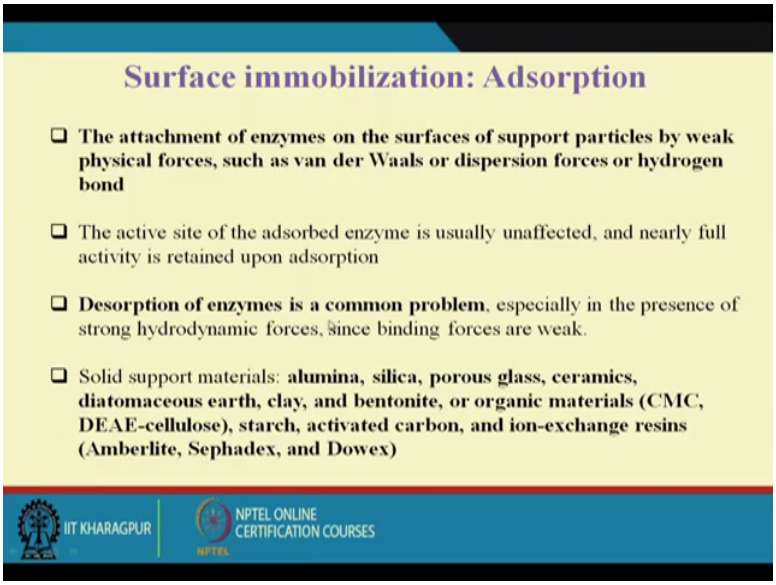

The desoroption of enzyme is common problem because as I told you that if you increase little bit higher force your enzyme will detach from the solid matrix specially in the presence of strong hydrodynamic force since binding force is very weak. Now solid substrate that is used for this purpose Alumina, silica, glass, ceramics, there are so many examples we have here.

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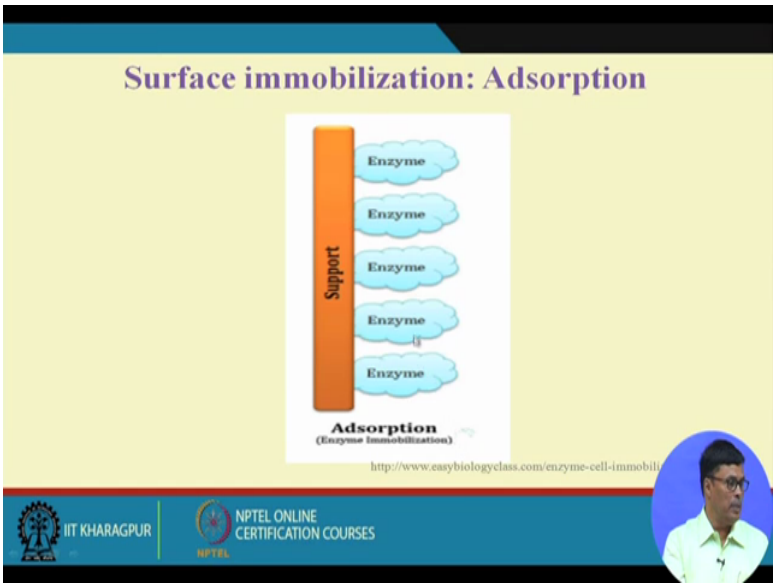

Now this is how adsorbed enzymes adsorbed in the surface like this. they simply added on the surface of the solid matrix.

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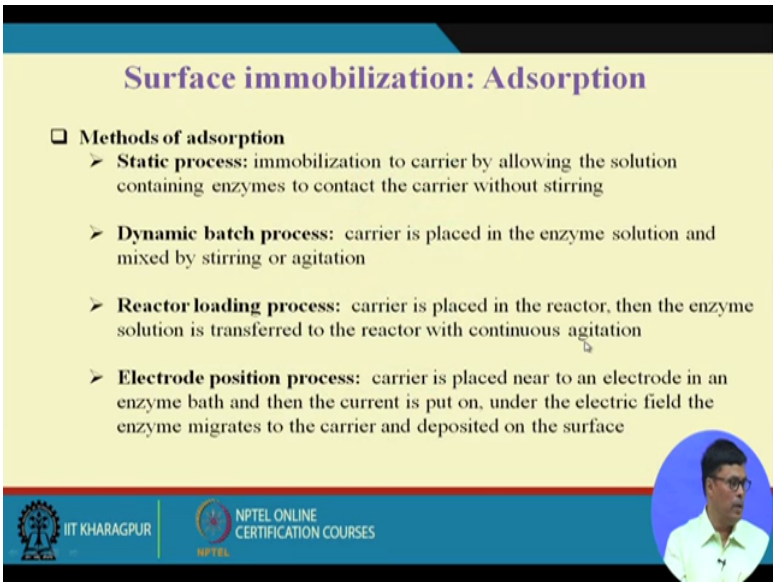

Now the method of adsorption we have several. We have static process, we have dynamic batch process we have reactor loading process we have electrode position process. Now static process we have immobilization to carrier by allowing the solution containing enzymes to contact the carrier without stirring. This is the this is called static because we don't make any kind of stirring here dynamic I can I can I can simple give a example here.

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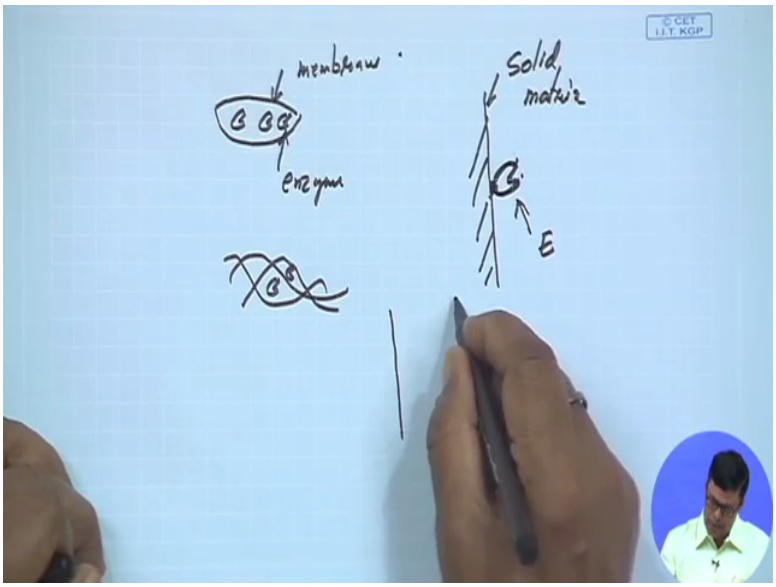

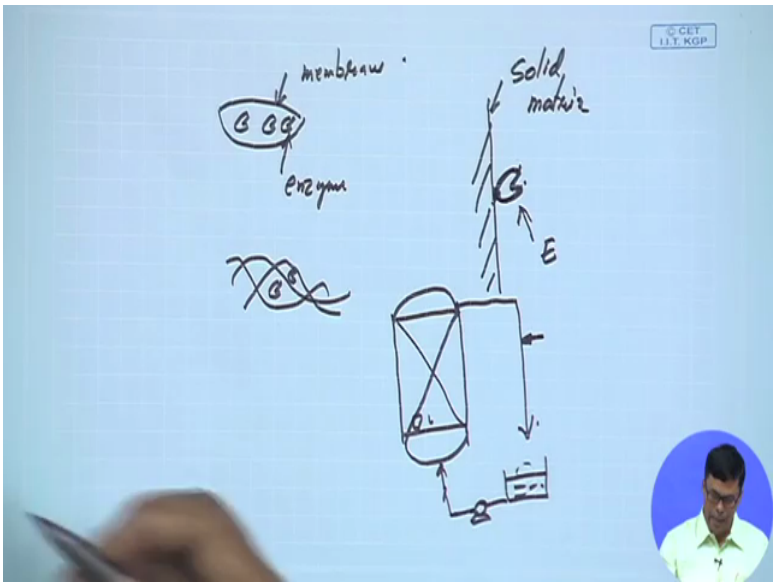

Suppose this a column and and we feel this with solid matrix here we can put different solid matrix and pass here suppose this is a enzyme solution we have enzyme solution and here we can have a pump with the help of the pump you can you can drawn the liquid in and and we can collect and again you can like this you can do the immobilization and recycle back again and again.

And until and unless your whole whole the solid start produce saturated with the with the enzymes and when we find the activities constant here and here the activity is constant that means your solid matrix is totally saturated with the enzyme so this is the this is the kind of here we don't have any kind of mixing

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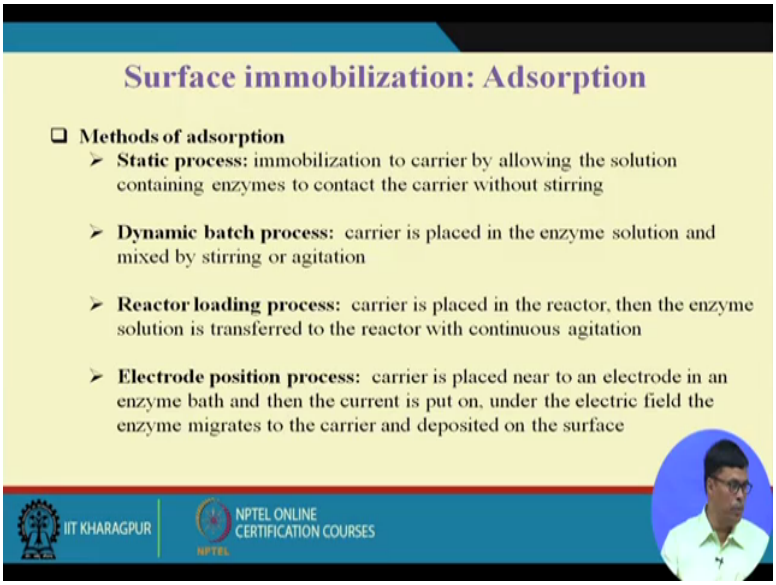

Just we circulate that thing dynamic batch process the carrier is placed the enzyme solution and mixed by stirring or agitation this is called dynamic batch mixing.

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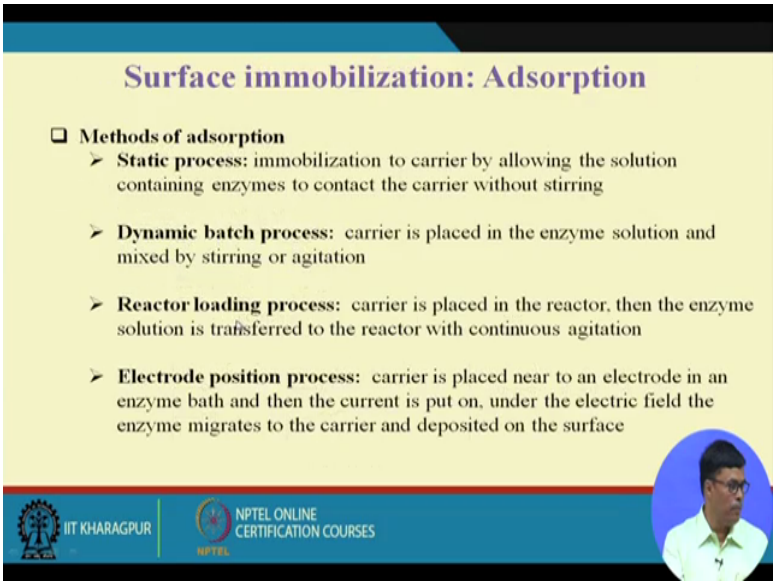

Now in case of the reactor loading process I I I I explain you carrier is placed in the reactor then enzyme solution transfer to the reactor is continuous agitation the electrode position

process the carrier is placed near the near to an electrode in an enzyme bath and then the current is put in under the electric field the enzyme migrate to the carrier and deposited on the surface because you know.

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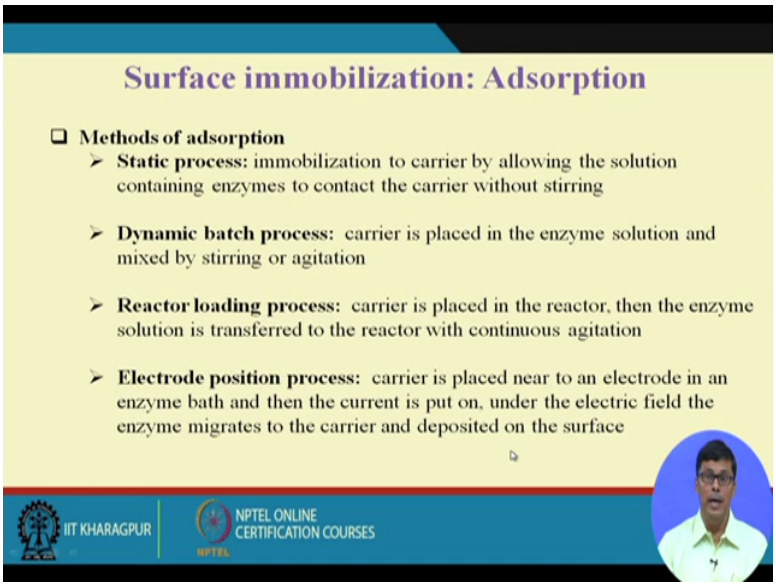

When when your enzyme carries some kind of charges you know that then your suppose you are your carrier has positive charge and your enzyme has negative charge so the electro static attraction is there but if you don't do that if you use as a electric field and and you ionised that that you know that the positive ion will attracted to the cathode negative ion will attracted to the so opposite charge they will attracts.

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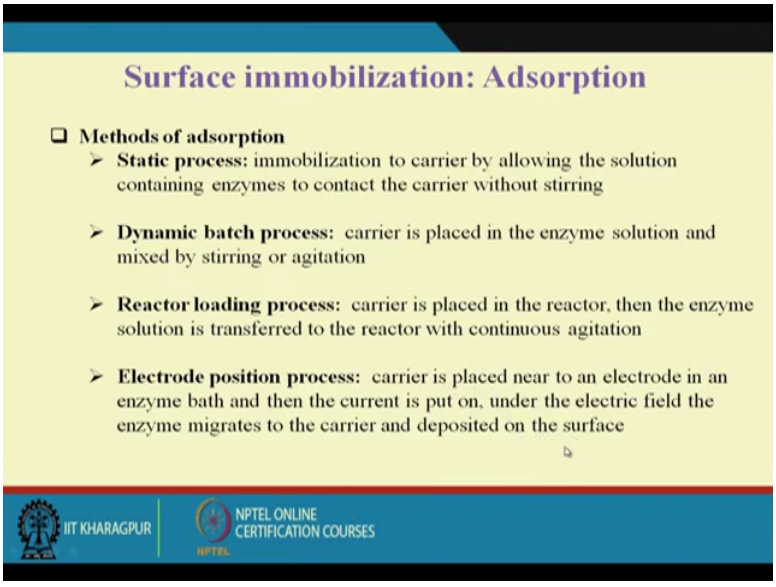

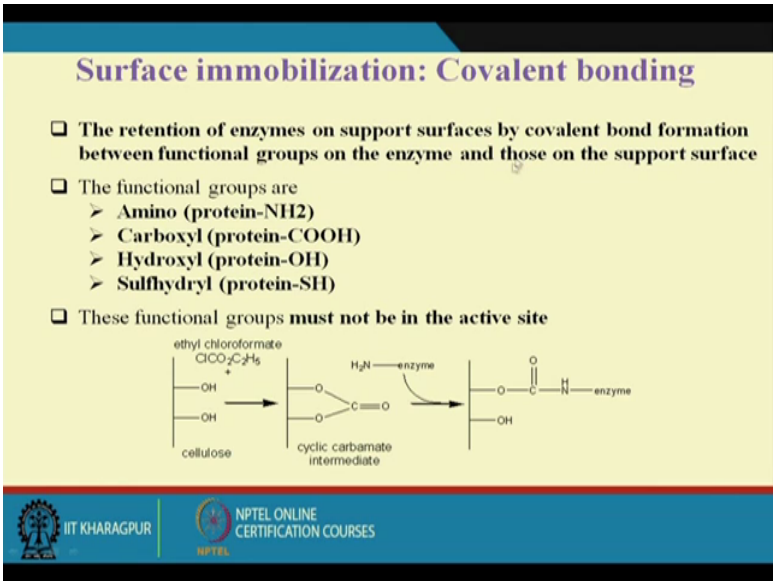

So this is how deposited on the surface now another I told you strongest bond is the covalent bonding but this is the very costly and and one thing I want to point out that during this process we lost lot of activity of the enzyme because because we we treat it with the with the solutions so lot of activities loss the functional groups are amino carboxylic.

Then hydroxyl and sulfhydryl this are the different functional group involved in this immobilization process now here I have taken the example of cellulose and in presence of ethyl chloroformate that that it gives us change in cyclic carbamate intermediate then we put the enzyme enzyme has a free amino group and this amino group will bind with a solid matrix like this so this is the we call this is the electron electron sharing so this will be the strongest bonding.

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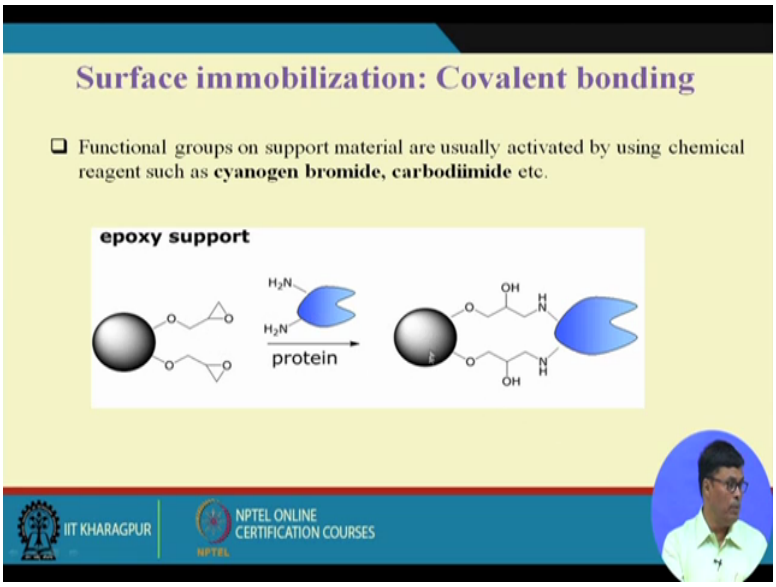

Now other examples are given here this is a this is like this how it is done and surface we can we can we can do this immobilization on the surface like this.

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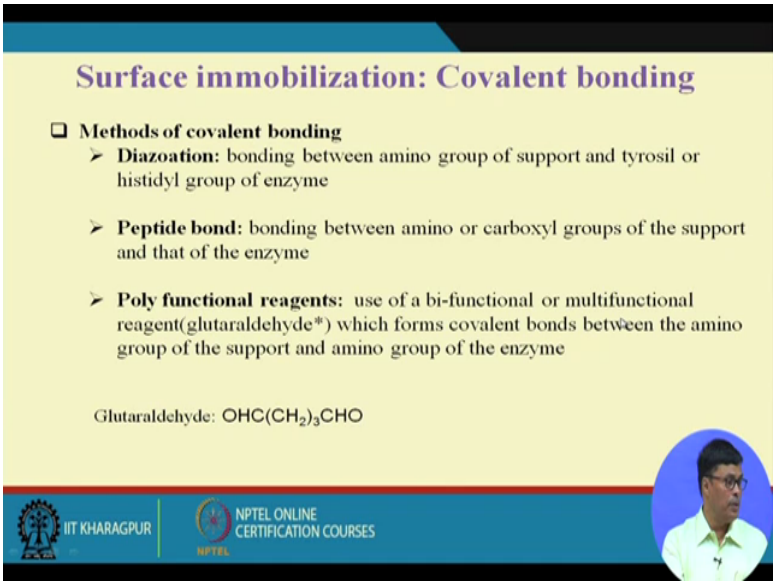

And now there are different type of reations we have we have diazoation then peptide bonding then polyfunctional reagents there are bonding methods of the covalent bonding there are stable.

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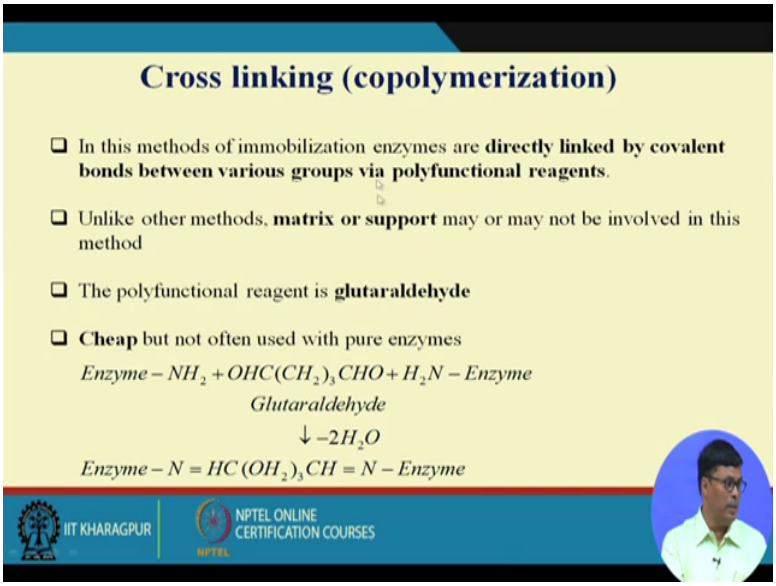

And another thing that is important is the cross linking because the cross linking may required matrix may not required matrix its directly linked by covalent between the various groups of the (())(27:08). I can give the example suppose this is the enzyme.

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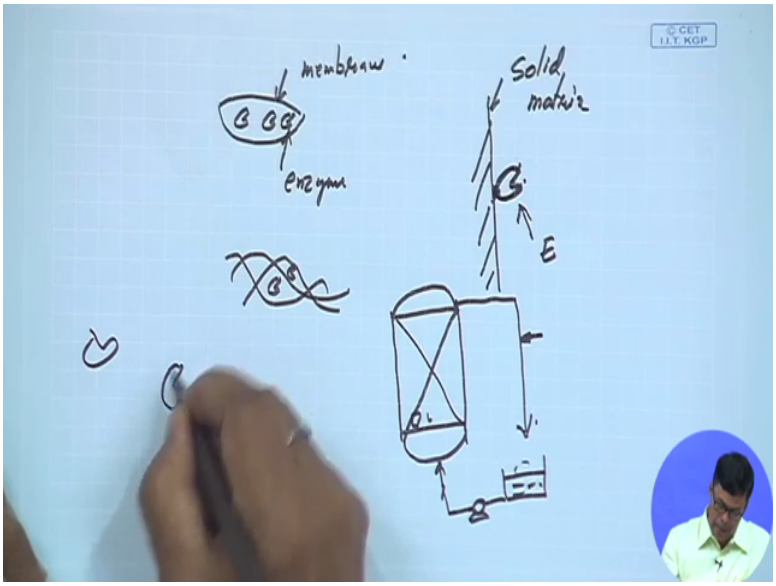

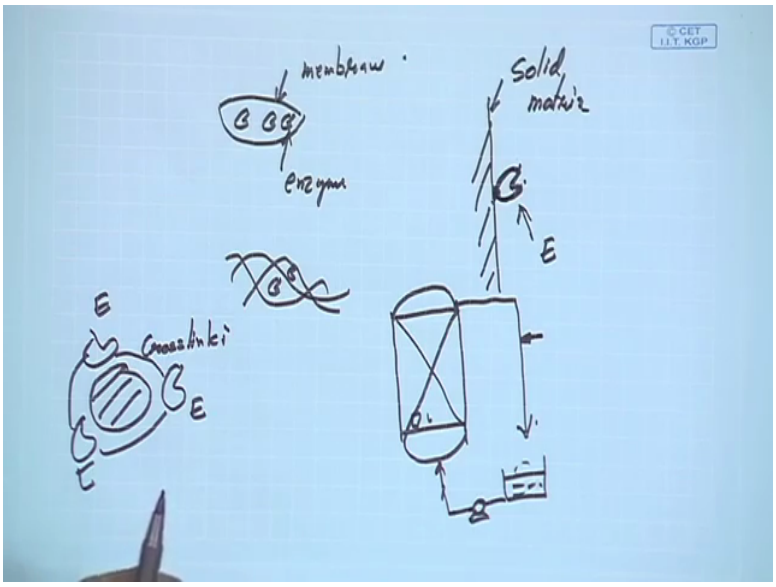

And this is this the enzyme so it can be it can be the crosslinking that this is the cross linking isn't so this is the enzyme this is the enzyme this is the enzyme so they cross linked with each other now if we if we put a solid matrix inside this then the enzyme rule embedded on the surface of the solid matrix enzyme will embedded on the surface of the solid matrix that is so this is can be done in presence of the solid matrix it can be done in absence of the solid matrix I have given the example here.

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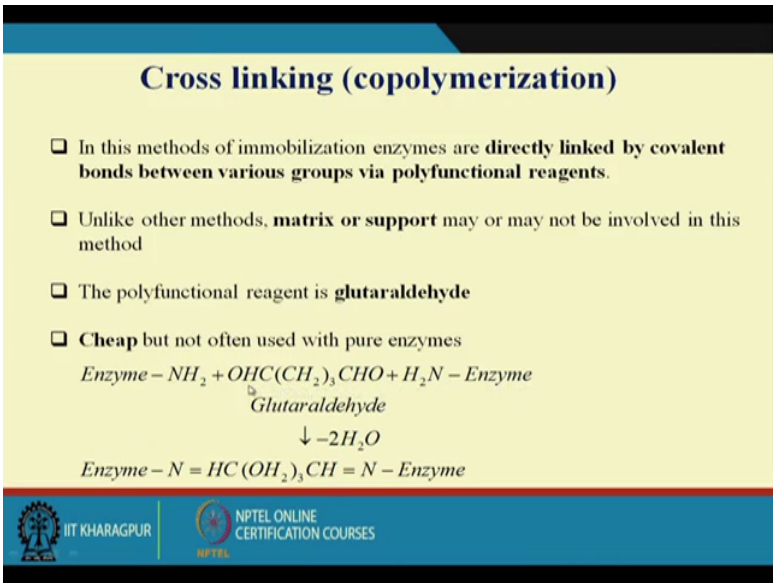

This is the this is the enzyme and this is the glutaraldehyde Glutaraldehyde to aldehyde groups and they are bind together this with this group like this I have given the example here.

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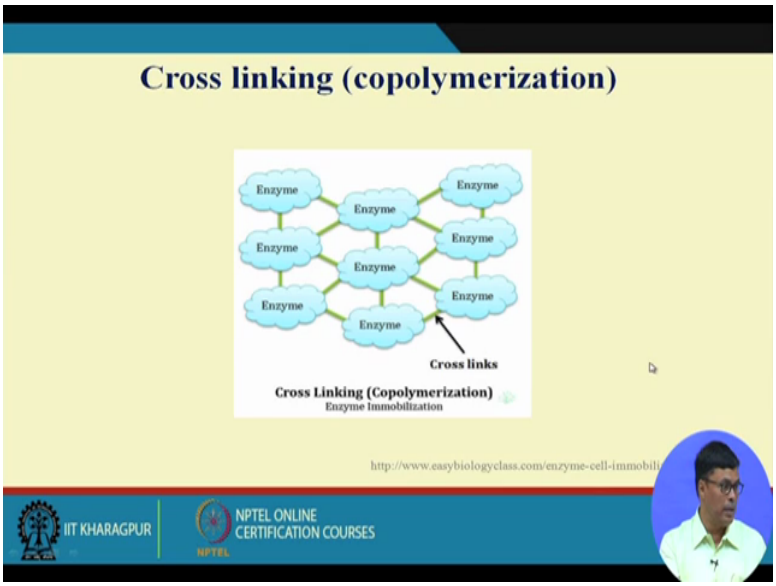

And this is how the cross linking takes place.

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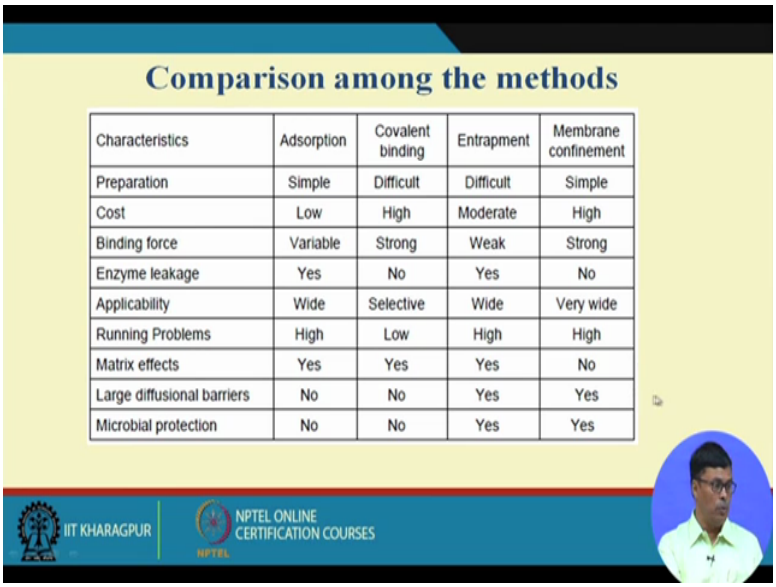

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

And finally I I told you that we have different type of immobilization take next and question comes how we select that which technique is good for this our immobilization of the enzyme then we we have certain characteristics preparation, cost, binding force, enzyme leakage, applicability, running problems matrix effect, large diffusion barrier and microbial protection now when we select the enzyme that you know process.

The immobilization technique first you have to you have to select what should be the characteristics of your solve of the solid matrix or immobilization technique that we have whether you are looking for easy preparation process whether you are binding force will be when the small whether it has large applicability.

So on the basis of your desire you can choose the immobilization technique. So this is all about this lecture I I I in the next class I shall discuss about the kinetics of the this immobilization enzyme technique. Thank you very much!