Aspects of Biochemical Engineering
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Lecture - 27
Immobilization of enzymes – I

Welcome back to my course industrial the aspects of biochemical engineering. Now, but today in this lecture I am going to cover a new topic that is the immobilization of enzymes. And the last couple of lecture I try to concentrate on enzymes, and we discussed the theoretical part of enzymes that what is the enzymes, and how you can determine the activity of the enzymes, then how the substrate enzyme interaction take place. And also how you can find out the volume of the reactor for getting a desired amount of substrate, and also we also discussed about the enzymatic elevation.

Now, immobilization enzyme has lot of industrial applications actually. Because we know that a major drawback of this enzyme is the stability of the enzyme because the enzyme cannot be stable for longer period of time. The immobilization is the one of the techniques through which we can improve the stability of the enzyme for a longer period of time; and not only that because when you talk about any kind of a enzymatic reaction, after the reaction is over, the enzymes remain as the impurities in the reaction mixture. So, after the reaction is over you have to remove the enzyme from the reaction mixture, so that is another problem that we have and that is why that when you use the immobilized enzyme, so you do not have that enzyme in the product much of the enzyme that is not present in the product. So, your purification problem that will be little bit easier.

And not only that that you can use the enzyme for longer period of time. Because in case of normal enzymatic reaction, after the reaction is over you have to the enzyme cannot be reused again; but in case of immobilized enzyme, you can reuse the enzyme again and again, until unless the activity declines. So, this lecture we will be deals with particular this immobilized enzyme process.

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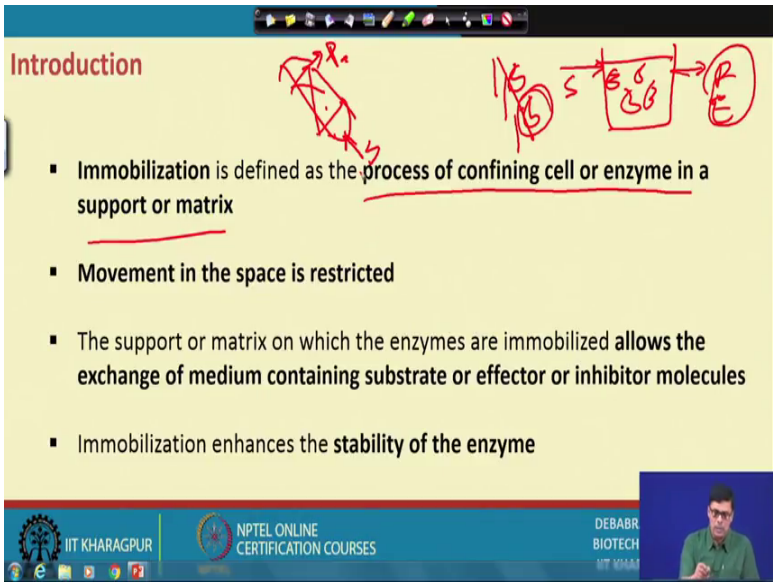

And first thing that I want to highlight what do mean by immobilization. Immobilization defined as the process of confining of cells or enzyme in a solid support. What do you mean by that? Suppose this is solid support, am I right, and your enzyme that is that immobilized. So, when suppose this a solution and the enzymes are there in a solution. So, they are freely moving, am I right, but when it is fixed on a solid matrix, their movement is arrested they are not moving they cannot move.

So, the advantage is that so suppose you have a column here, you have a small column you and you immobilize the enzyme, you can pass your substrate here, and you can get your product in other end. But here when you pass your substrate and get the product the with the product, you will get enzyme also. Here you will not get much of enzyme. So, basically that immobilization defines as the you are fixing the enzyme on a solid matrix and movement of the space is restricted that is the major thing that we have.

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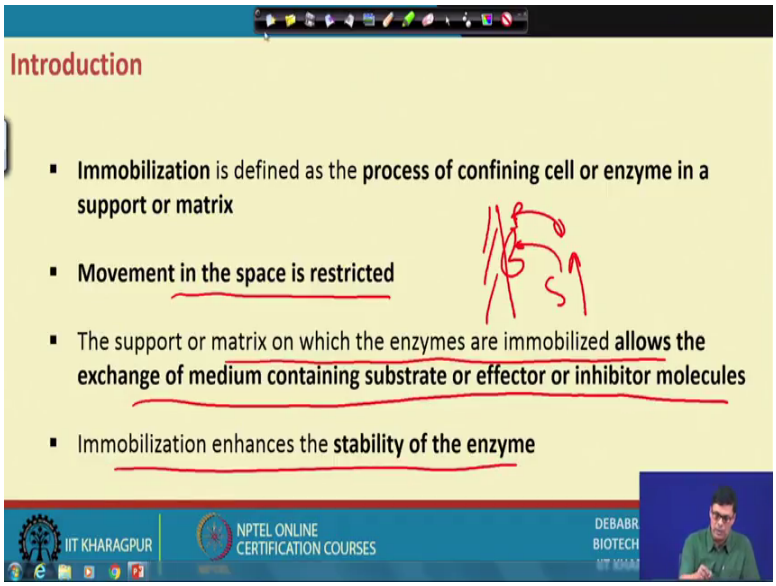

And the support or matrix on which the enzymes are immobilized allows the exchange of medium containing substrate or effector or inhibitor molecule. So, what I want to tell that suppose your enzyme is immobilized here, and your substrate is here in the bulk, so this is to diffuse here. And then and only then your reaction will take place and after the product form the product will come to the bulk. So, this is the process that will take place in case immobilize system. Immobilize enhances the stability of the enzyme, I already discussed this increases, thus stability of the enzyme.

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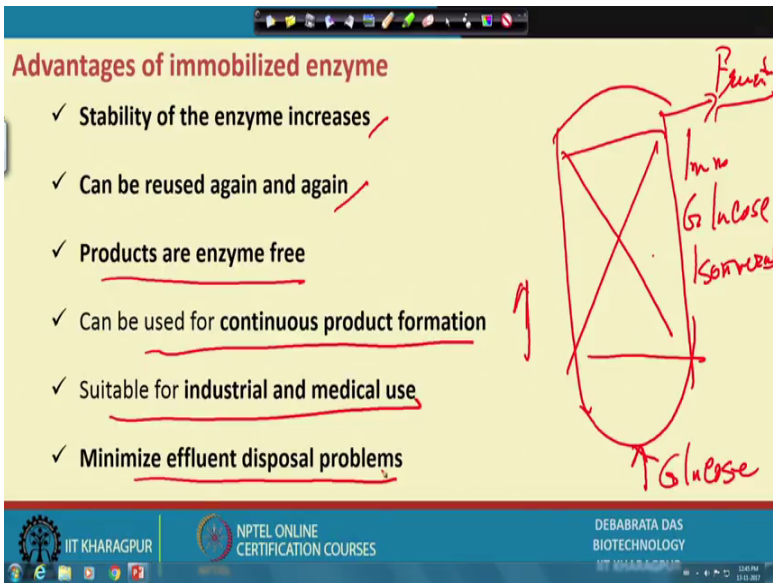

Now, advantage of the immobilized system that first with the stability increases that already we pointed out can be reused again and again that also I pointed out. So, suppose in a big column, so you can put your immobilized enzyme here, and here you pass suppose you want to produce glucose, glucose to fructose, the glucose isomerase enzyme glucose isomerase, so you immobilized that immobilized glucose isomerase here you will get the fructose.

So, now that with this you can you can operate it continuously and until unless your reaction declines. And products are free from enzymes; and can be used continuous product formation; suitable for industrial and medical use. How it is medically used, because we know that due to some genetic disorder that sometimes some of us unable to produce some kind of desired enzyme, so that enzyme we shall have to take from outside for our survival. Now, if you take the enzymes in the form of tablets, then you have to take lot of tablets.

Now, suppose some enzymes if you keep it immobilized, and put in the bloodstream, so you can use that enzyme that for longer period of time you do not have to take the enzyme again and again. So, this is one of the important application in the we have in the medical sector, medicinal sector. And the minimized the effluent disposal problems, since it does not have your enzyme in the product, so your disposal problem also will be reduced.

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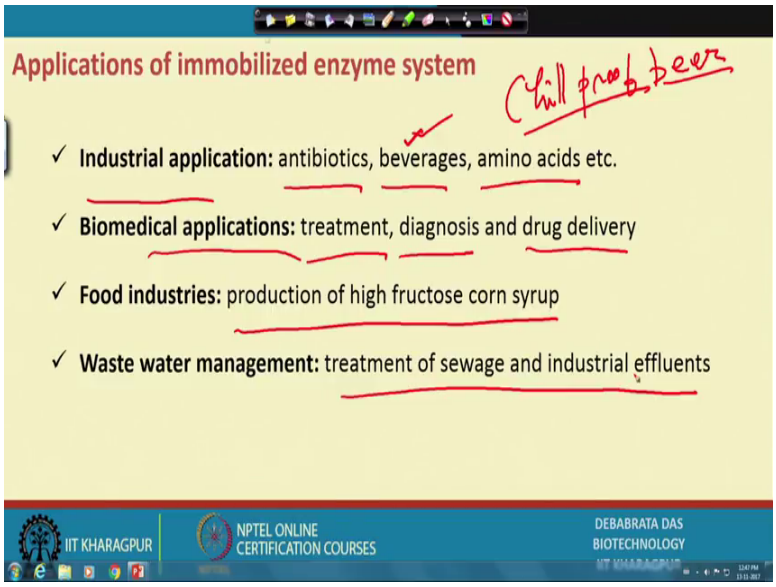

Now, application of immobilized system that examples if you look at the industrial application, we have antibiotics beverages and the amino acid because. I can give a very typical example that you know chill proof beer I do not know whether you have heard that chill proof beer. What do you mean by chill proof beer the beer is usually sold on the chill condition.

And we know the beer is kind of beverage that that contains lot of proteins. Now, since we need served at low temperature there is a every possibility that if there is a protein bigger molecular protein it may precipitated out. Now, if it precipitated out it will give some kind of haze in the in the solution that is undesirable. So, by this is lot of application, this is one of the application that we have in the beverage industry.

Then medicinal application. I told you treatment and diagnosis. Diagnosis means that you know that suppose particularly during the athlete competition that you know if you want to find out that whether the athlete they had to take it any kind of stimulant. So, we can have a sensor that you know biosensor, if we have we can from the biosensor, we can easily detect that whether they have taken any kind of stimulant, drug delivery system which can be used. Food industry it is used for the production of high fructose corn syrup.

This is mostly used in the confectionery industry. And waste treatment process and sewage and industrial particularly trickling filter are that is largely used in the industry that for the removable of the soluble organic present in the in the waste water.

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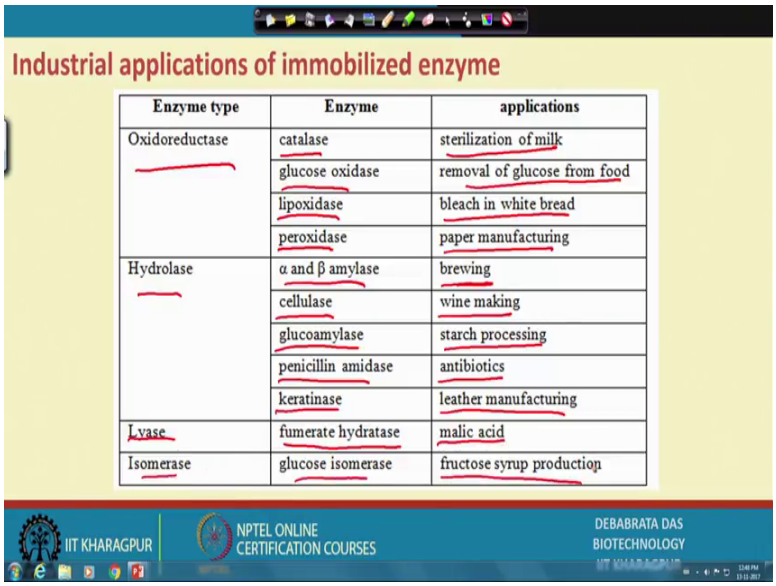

Now, lipoxidase that bleaches the white in white bread the bread and peroxidase paper manufacturing industry. Hydrolase we have alpha beta amylase that is used in brewing industry. Cellulase wine making, the glucoamylase the starch processing, penicillin amidase that is the antibiotics industry, keratinase leather manufacturing industry. Lyase, fumerate hydratase that the malic acid, and isomerase glucose isomerase for fructose syrup production. These are the different industrial applications we have.

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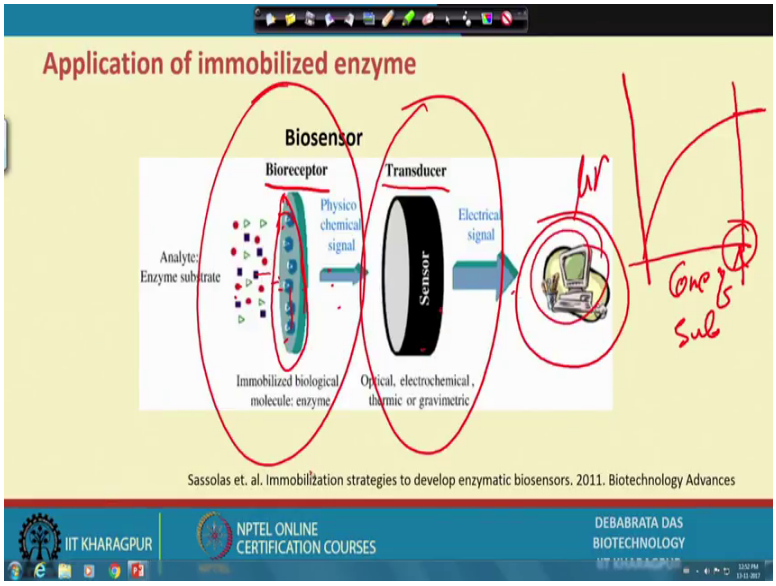

Now, as I pointed out that one important application that we have is the biosensor. Now, question come how biosensor they are produced. Now, here in this picture, you will find that that two part is there one is this is bioreceptor, and this we call molecular recognition unit, and this is called transducer. So, you know it has it divided into two different parts. One is molecular recognition unit; another is transducer. The molecular recognition unit that mean part for to recognise the molecule.

As for example, suppose glucose oxidase we have now glucose oxidase in presence of glucose they will immediately they will react and produce degrade glucolactone. So, you know that you know that reaction is there you know that and that reaction that chemical change we can converted to the electrical signal that is with the help of transducer. And this is mostly this may be of different types. The mostly we have two different types, one is potentiometric, another is amperometric metric. The potentiometric means with respect to change of voltage and amperometric means with the change of current that we can easily find out what is the change that take place because if what a and that we correlate with the concentration.

I can give you very simple example. Suppose this is micro volt and this is the concentration of substrate may be glucose that we have. So, it is like this. So, the major problem is that that after this concentration this attains the plateau that is that is the major problem; that means, it is effective up to this concentration. After this concentration is not effective, so that is that is the major problem when you use as a biosensor your concentration of the sample, concentration of the substrate should be as low as possible.

So, if you if you the concentration is high, then you shall have to dilute significantly. But the advantage is that you can you can analyse n number of sample, and instaneously you can do that, it will not take any time. But if you go for any analytical technique you have to prepare the sample, you have to add different chemicals then you have to put in the spectrophotometer, it takes lot of time, but this is the major the frequency of analysis that increases drastically.

So, this is how it is done. This is bio receptor; and this is transducer. And this is how we can detect that you know that you know direct we can detect that how much we correlate that how much that the first you can you can you can see that here that that the biomolecules they are immobilized on this. I can give the example. If the enzymes are

immobilize on the surface, then enzymes are very specific with respect to substrate when the reactor is converted into different compound, then when this reaction take place it will convert into electrical signal, and this electrical signal will be recorded in the our recorder.

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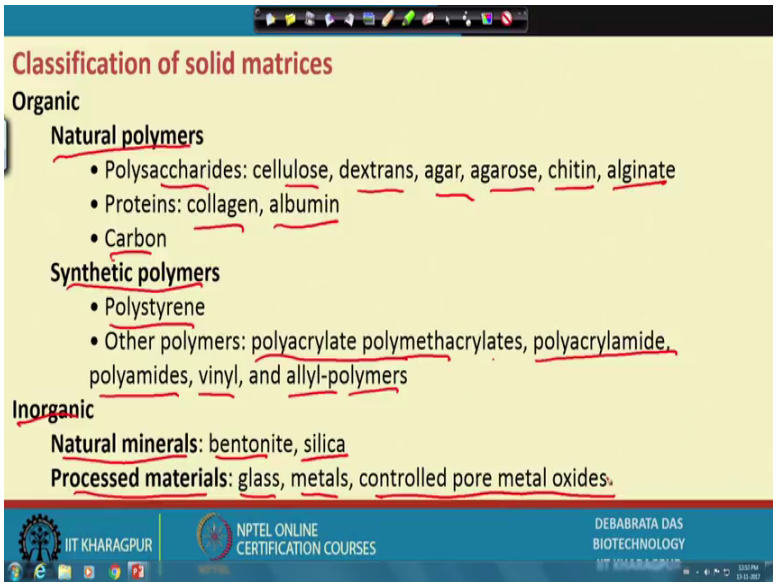

Now, classification of solid matrices, solid matrices that is used for immobilization purpose because different type of solid matrices we considered, one is natural polymer then synthetic polymer then inorganic we have natural minerals and process minerals. Now, what is the natural polymers, we have polysaccharides like cellulose, dextrans, agar, agarose, chitin and alignate.

Protein, we have collagen largely use particularly I work with IIT, Delhi; and to we have we use the collagen membrane for the immobilization of glucose isomerase enzyme, albumin and also that carbon. The synthetic polymer we have polystyrene, and other polymer we have polyacrylate, poly methacrylate, the polyacrylamide, the polyamide and vinyl and allyl-polymers. Natural minerals are bentonite, and silica. Then process minerals are glass, metal, controlled pore metal oxides. This is the different examples of the solid matrix.

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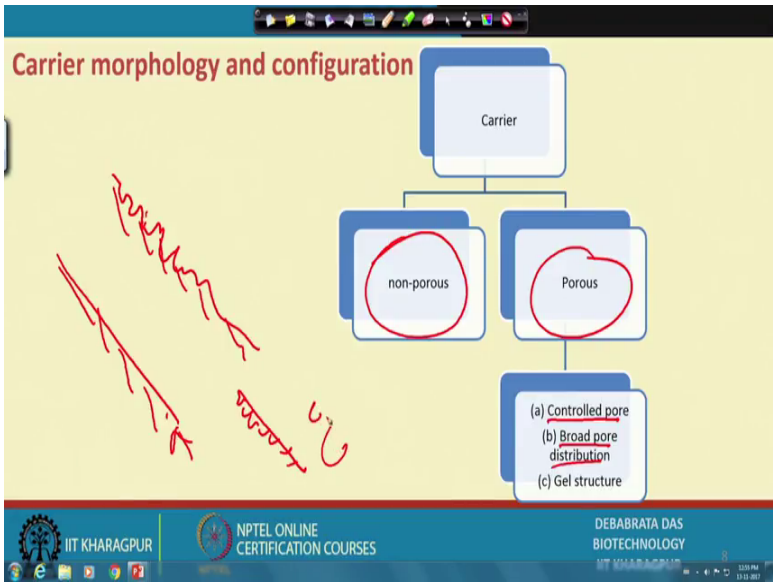

So, when we when we talk about any kind of solid carrier, broadly it can be divided into two types, one is called non-porous, one is called non-porous and another is porous; that means, you know that suppose that this is solid matrix. So, it does not have any pore, but this solid matrix may be having this kind of pore. So, you know that this is this is this is how ports inside the pore that you know that some pore is there inside the solid matrix. So, this is non-porous; this is porous.

So, in porous also these pore may be of two type, one is controlled pore; another is broad pore distribution that means, when the pore size is uniform, suppose your pore size is uniform like this then this is controlled pore, so that is the obliviously that will very costly. And broad pore distribution means pore size are different some size is bigger some size is smaller like this inside this solid matrix, we have that different size of these pores.

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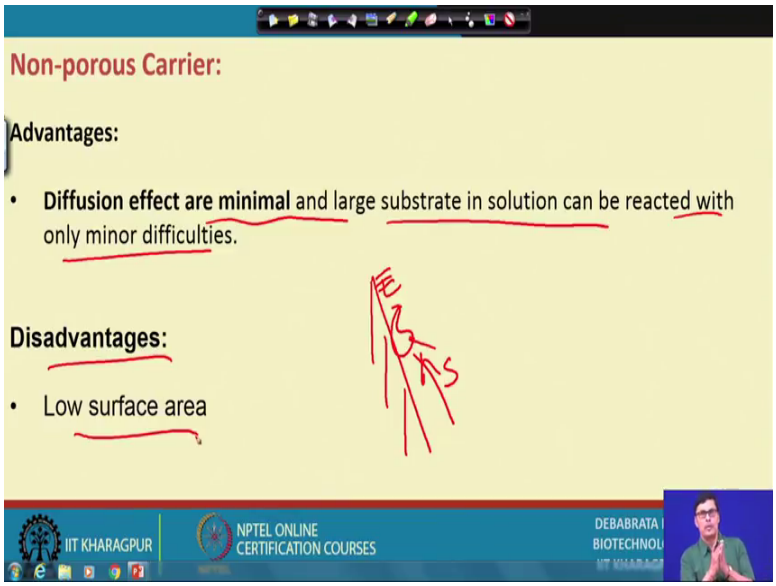

Now, non-porous what is the advantage that diffusion effect is minimal, because it is very obvious because suppose this is this is solid matrix and enzymes are immobilized on the surface. So, when your substrate is going that is freely interact with enzymes, this is the enzyme this freely interact with the enzyme. The diffusion effects are minimal and large substrate in solution can be reacted with minor difficulties. Disadvantages are low surface area because since the surface is smooth that surface area will be very low.

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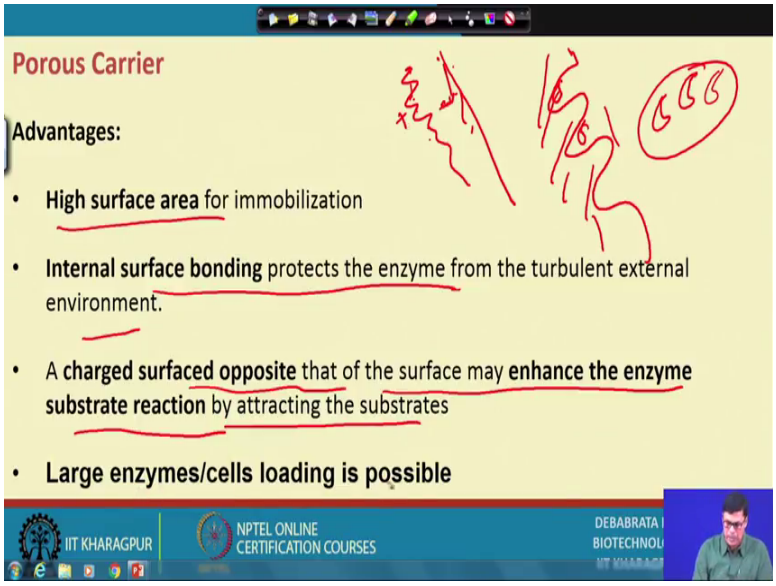

Now, porous has lot of advantages, because let me say is tell you the first is the high surface area. This is the major, major advantages the so since there is you have porous surface, so with that porous surface that you know that we have as compared to smooth surface this will obviously, that this porous surface has lot of area. So, more enzyme if more surface area more enzyme will be immobilized on the surface. The internal surface bonding protect the enzyme.

Now, suppose this is the this is the this is this is how your solid matrix. Now, if your enzymes are here it gives some kind of protection because it embedded or when you talk about the suppose encapsulated the enzyme, so it has it has the some unclipped when there was kept protect the enzyme. The internal surface bonding protect the enzyme from the turbulent or external environment.

And a charged surface opposite that of the surface may enhance the surface enzyme substrate by attracting the substrate. Suppose, the here we have if you have the positive charge and your substrate has a negative charge obviously, the interaction between the enzymes interaction with the with the substrate with the enzymes will be more, because your matrix charge is opposite to the substrate. And last large enzymes are cell loading possibility since pore size is more, so it is obvious that you know loading also will be more.

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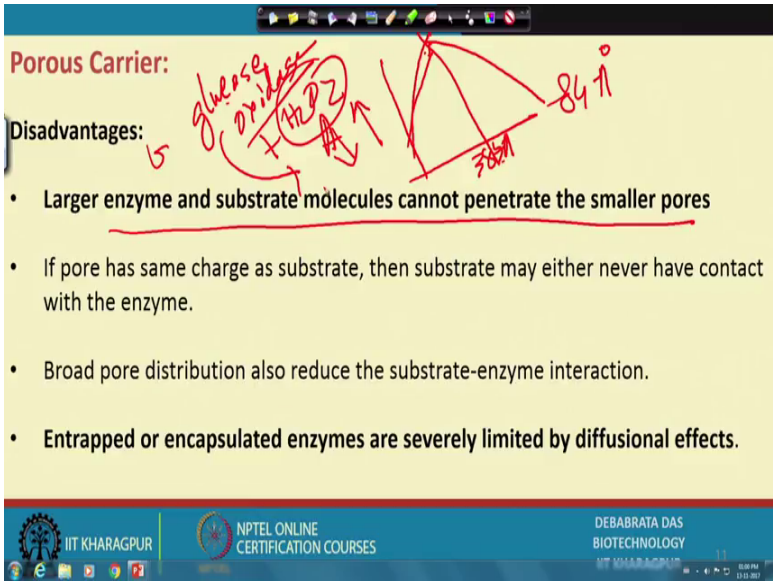

Now, disadvantages lot of disadvantages also there. Larger enzyme and substrate molecules cannot be penetrate in the smaller pores, but there is a rule of thumb I can tell you that I will just show you at the end, there is the enzyme called catalyst enzyme. What is the what is the function of the catalyst enzyme? It degrade the hydrogen peroxide to water and oxygen, am I right. And this is largely present in the aerobes, because aerobic organisms. Now, in case of anaerobic organism particularly (Refer Time: 19:11) that will obligate in the analogue they do not have this catalyst enzyme. And since they do not have this catalyst enzyme, so when oxygen goes to the their system it produce hydrogen peroxide and super oxide and this super oxide has oxidizing property.

So, since they have oxidizing property, they will change the characteristics of the bio-molecules. And if they change the characteristic of the bio-molecules then the activity of the enzyme activity of the cells or enzyme will be will be lost though this is the major problem that we have with this pores. So, so large molecules cannot penetrate the, so what problem will happen let me tell you that when you when you the rule of the thumb is that the size of the pore should be double the size of the enzyme. Suppose in the size of the enzyme is 84 Armstrong unit, so it should be 2 into 84 Armstrong unit that is 168 Armstrong unit should be the pore size.

But in case of catalyst, though it is round about this 84 Armstrong, but we find that if you if you plot like this activity of the enzymes with this, we will find to keep on increasing with the pore size. And this pore size is something similar to 364 or something like this. Now, question comes why because it required some no I have I told little bit different that this is I am talking about the glucose sorry the glucose oxidization enzyme.

Now, in case of glucose oxidization enzyme when it acts on glucose, it produce hydrogen peroxide. And this hydrogen peroxide should be degraded by the catalyst enzyme now hydrogen peroxide has the pore size 84 Armstrong unit. So, when you when you when you plot the activity of the glucose oxidant, you find it is keep on increasing, but maybe at 384 or 87 the Armstrong we get the we get the maximum activity. But the reason is that that this catalyst has the size much higher than this, and we find that this side is related to the size of the catalyst enzyme.

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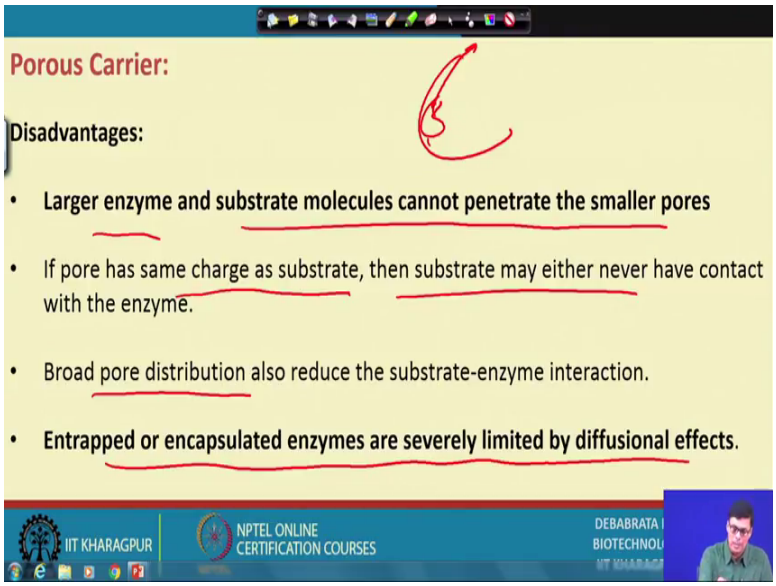

So,. So, you know large molecule are substrate cannot penetrate, this is the major problem that with the porous solid matrix. Now, if the pore has same charge as substrate then also interaction will be less pore. Broad pore if a pore size is more then also this is undesirable that there is every possibility that there enzyme substrate interaction will be very less. Entrapped or encapsulation are severely limited by diffusional effect. So, this is this is the diffusional effect means if pore is there then substrate has to enter into the pore, so that naturally your diffusional problem in this case will be very stringent.

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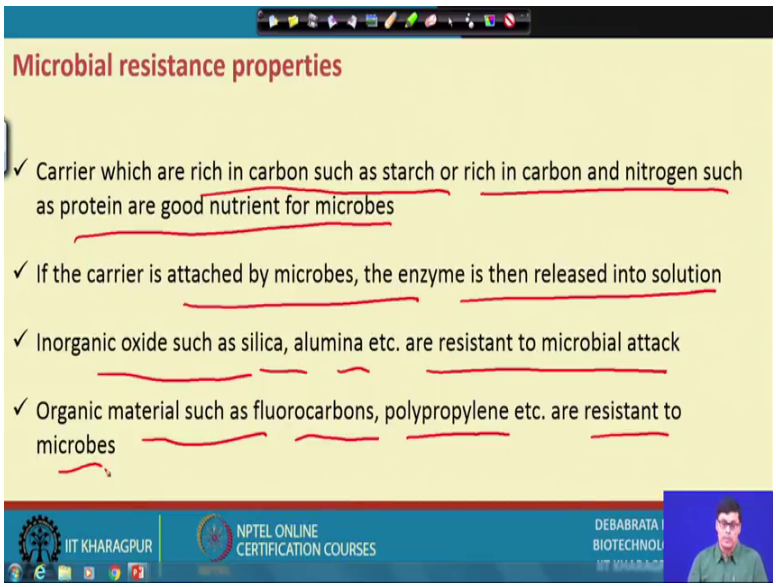

Now, microbial resistance property that is very important that is if you look at the carrier which are rich in carbon such as starch or rich in carbon and nitrogen such as protein are good nutrient to the microbes. Now, if these molecules are present as a solid matrix, then the every possibility that your microorganism can grow and spoil this solid matrix. If the carrier is attached to the microbes, the enzyme is then released into the solution. Now, inorganic oxide like silica, alumina are resistant to the microbial attack. Organic material such as fluorocarbons, polypropylene etcetera are resistance to the microbes. So, microbial resistance property of the solid matrix that is also plays a very important role when we select this, what should be this solid matrix we should use.

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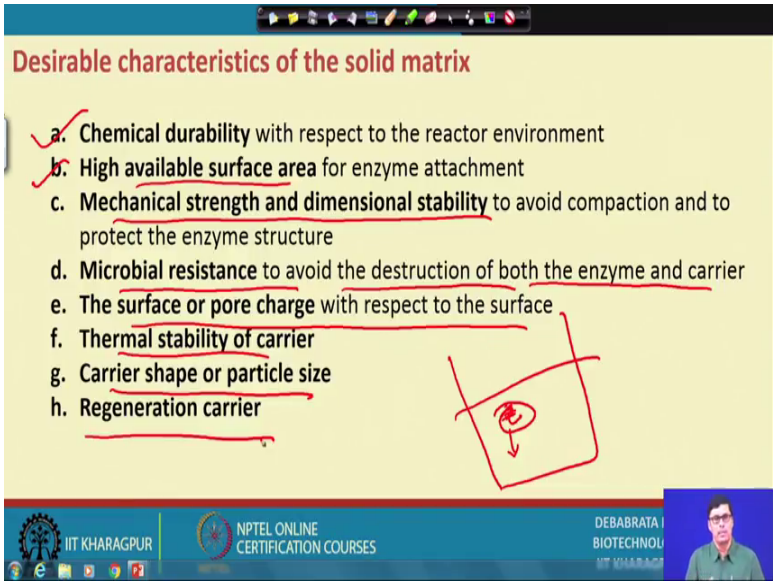

Now, what the question come what should be the characteristics the desirable characteristics of the solid matrix. First it should be that you know chemical durability. What do you mean by that, that you know that suppose you want to carry out the reaction a to b, the b is the new product a also a chemical product. Now, it should withstand both the a and b; otherwise if degraded then that is undesirable. Then high available surface I already discussed just now more surface area more there will be enzyme that will interact. And if more enzyme is there more it will interact with the substrate and give the product.

Mechanical strength and dimensional stability; this is very important because as you know that when suppose there is a solution and when you do the immobilization that

what you do that in the solid matrix your enzymes are immobilized. Now, what is the characteristics of the solid matrix, they will settle down am I right. So, you if you want to have more interaction with the substrate, you should have mechanical stirrer. And if there is a mechanical stirrer your solid matrix should withstand this mechanical pores mechanical strength and dimension stability.

Then microbial resistance this is obviously, to avoid the destruction both the enzyme and carrier. And surface and pore charge with respect to the surface you know the pore charge should be if preferably should be opposite to the substrate, so the more enzyme substance interaction take place. Thermal stability particularly we talk about the alpha amylase enzyme that usually act as the high temperature as high as 60 degree centigrade. So, if I if you select any kind of solid matrix for alpha amylase enzyme it should withstand that high temperature.

The carrier shape and particle size that plays very important role. We know the spherical size has more surface areas compared to other, other solid material. The regeneration of the carrier that plays very important role. Suppose, we want to use any kind of solid matrix maybe it is very cheap, but we might be using for single time. But so here again when you that that is so and but some of the solid matrix I can give the example of platinum foil that is very expensive platinum is costlier than gold. But platinum foil you can reuse again and again then you know for longer period of time after, after when your enzymes are inactivated, you take it out and put in a 600 degree centigrade for 2-3 minutes all the organic material will burn out again you can use it is for immobilization purpose.

So, regeneration of the that solid material though they initially investment is very high, but you can reuse again and again. So, regeneration also place very important role.

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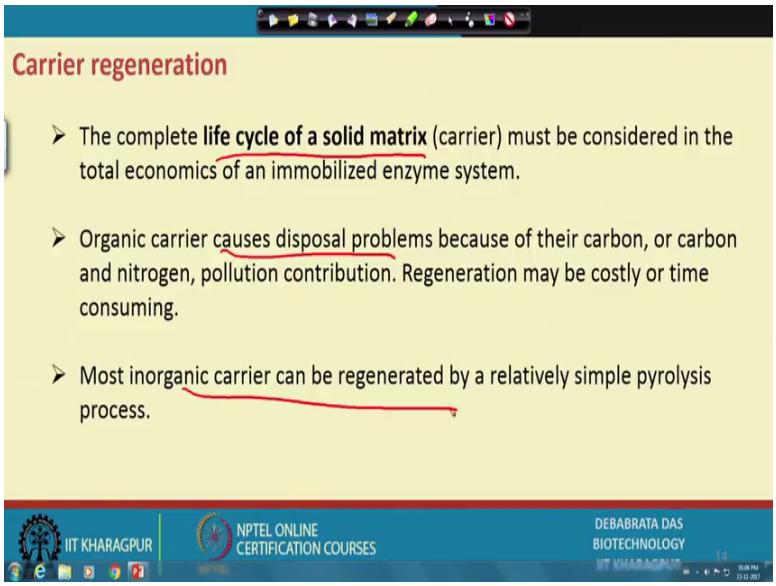

Now, carrier regeneration what I was talking about this is life cycle of the solid matrix is very important that how long you are going to use. The organic material causes the disposal problem that is very important, but inorganic carrier can be regenerated and relatively simple pyrolysis we can regenerate by with the help of simple pyrolysis process. I was talking about the by simple heating we can we can we can do that.

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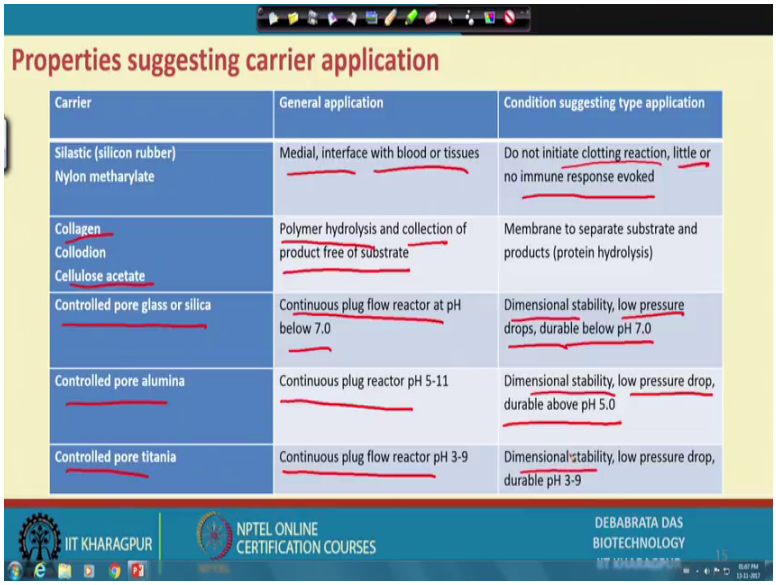

Now, this is the this is the properties are used to carrier applications that silastic, this is silicon rubber, and nylon metharylate that is medicinal interface with the blood and

tissues. And do not initiate the clothing reaction, little or no immune response evoked. The collagen, this is another cellulose acetate that is used. This is for polymer hydrolysis collection of product free substrate. Then controlled pore glass that is your continuous plug flow reactor at pH below 7; dimensional stability, low pressure drop, and duration durable between below pH 7. Controlled pore alumina, continuous plug flow reactor 5 to 11; dimensional stability, low pressure drop and durable about pH in above pH 5. Controlled pore titania, this is continuous plug flow reactor. So, these are the in the different properties of some carriers that has been reported.

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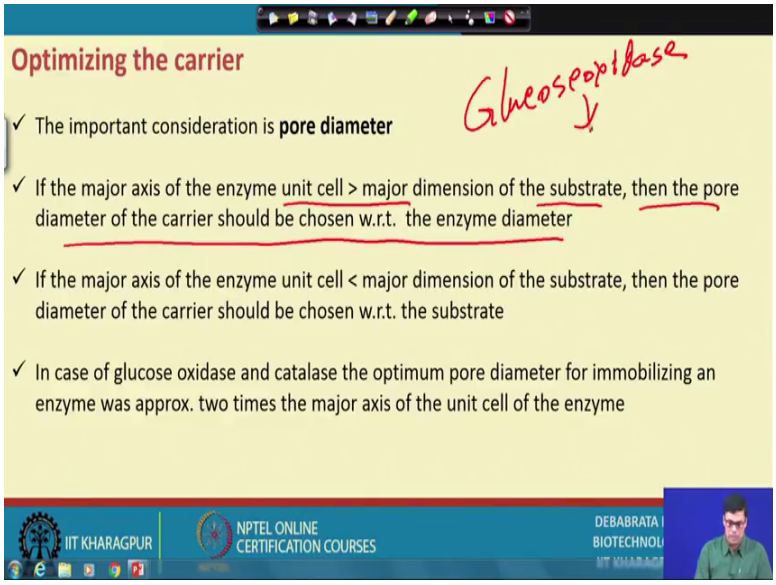

Now, optimizing the carrier that is the pore diameter as I pointed out that important consideration major axis of the enzyme the unit cell should be greater than the major dimension of the substrate, then the pore diameter carrier should be chosen with respect to enzyme diameter. I have I was giving the example of glucose oxidase that glucose oxidase enzyme. So, in this in this following example that we can find out that here.

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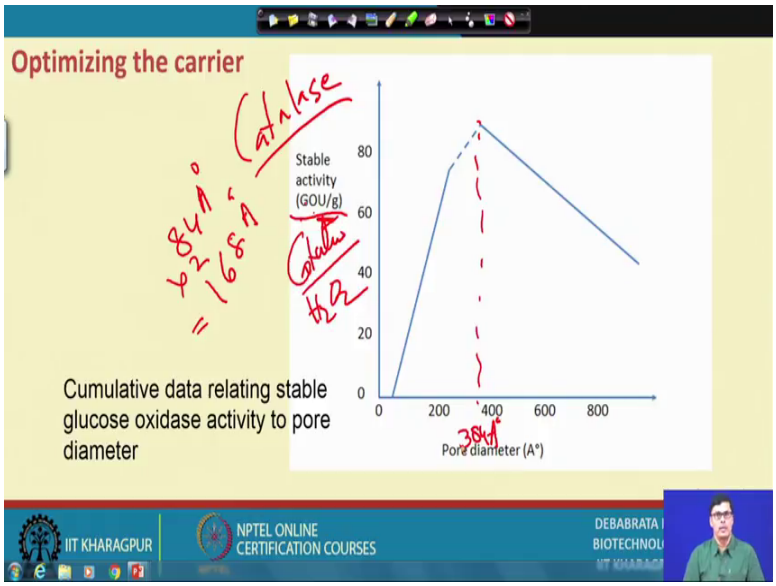

Here we have the this is the glucose oxidase enzyme activity. And this is the exactly what I am saying that this is around 384 or something like this Armstrong unit. And if you look at the size of this is 84 Armstrong. So, so this should be into by 2, it should be 168 am I right Armstrong, but it is the about 384. So, this is this is due to the reason is that that glucose oxidase will cannot work without the presence of catalyse enzyme.

Because why the without the catalysing enzyme it cannot work, because catalyse enzyme then the one of the product with the glucose oxidase is the hydrogen peroxide. And catalyse enzyme catalyse enzyme that degrade the degrade the hydrogen peroxide to water and oxygen. So, that is why the otherwise if you do not use the catalyse with the glucose oxidase, then the accumulation of hydrogen peroxide will take place and your activity of the enzyme will be lost. So, that is why we find in case of glucose oxidase enzyme about 384 or 380 Armstrong most suitable for the immobilization of the enzymes.

So, so in conclusion what I want to tell that immobilization is a is a very important technique through which we can use we can make proper use of the enzyme, because it has it has several advantages. It has some disadvantages also. The major advantage is that you can reuse the enzyme again and again for longer period of time. Your product is preformed enzyme, so your purification problem will be little bit easier, your disposal

problem also very less. But problem is that when we immobilize; that means, they are fixing the sale on the solid matrix the diffusion problem is the major problem.

Then question is the solid matrix because as soon as you immobilize that your if suppose your enzyme is soluble where as soon as the immobilize that it will become insoluble. So, you are switching over from homogenous system to the heterogeneous system, heterogeneous system major problem we have a told you that the diffusion problem. The diffusion problem we have to take into account. And characteristics of solid matrix I tried to discuss the characteristics of the solid matrix is that you should withstand chemically stable, it should withstand the it should have good mechanical power and regeneration characteristics and surface area should be as high as possible.

So, you know the different characteristics we have we have seen that solve that that solid matrix may be of two types one is pore, another non-porous. In porous we have a porous solid matrix have been greater surface area though there is a possibility of more immobilized enzyme. So, more enzymes are immobilized more will be the rate of reaction, so that is desirable. So, that is all for today that the next class I shall give some more information on the immobilized enzyme.

Thank you.