ENZYME SCIENCE AND ENGINEERING

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LECTURE-17
IMMOBILIZATION OF ENZYMES BY ENTRAPMENT

So continuing with the methods for immobilization of enzymes, we shall be talking today about the entrapment methods.

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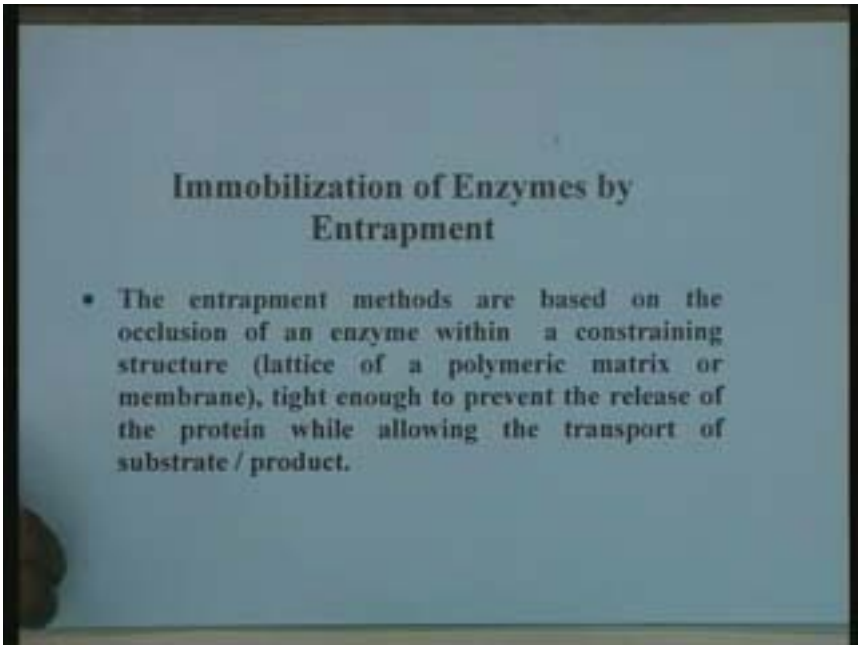

So far we have discussed various methods under the category of adsorption including ionic binding and metal salt linkage, cross linking and covalent bond formation. Entrapment methods are also a very large class of enzyme immobilization methods that are based on the occlusion of an enzyme within a constraining structure. That means the enzyme molecules are entrapped within a definite region of a space; it could be a lattice of a polymeric matrix or a semi permeable membrane. This matrix within which the enzyme is entrapped must be tight enough to prevent the release of the protein and allow the substrate or product molecule to pass through the boundaries. It means that when we have an enzyme which is entrapped within a gel matrix the gel should be porous enough so that within the pores it can retain the enzyme molecules which are large molecular weight in size of that of a protein but the porous structure must be such that the small molecular weight substrate or product molecules are allowed to pass through and therefore the reaction can be carried out. On the other hand if the entrapped preparation is

like a micro capsule which also consist of a semi permeable membrane boundary the permeability of the membrane must be such that the enzyme molecules are not allowed to be leaked out while the substrate and the product molecule can pass through easily.

Some of the important characteristics of these preparations is that only reactions involving relatively small substrate or product molecules can be carried out successfully using entrapped enzyme preparations.

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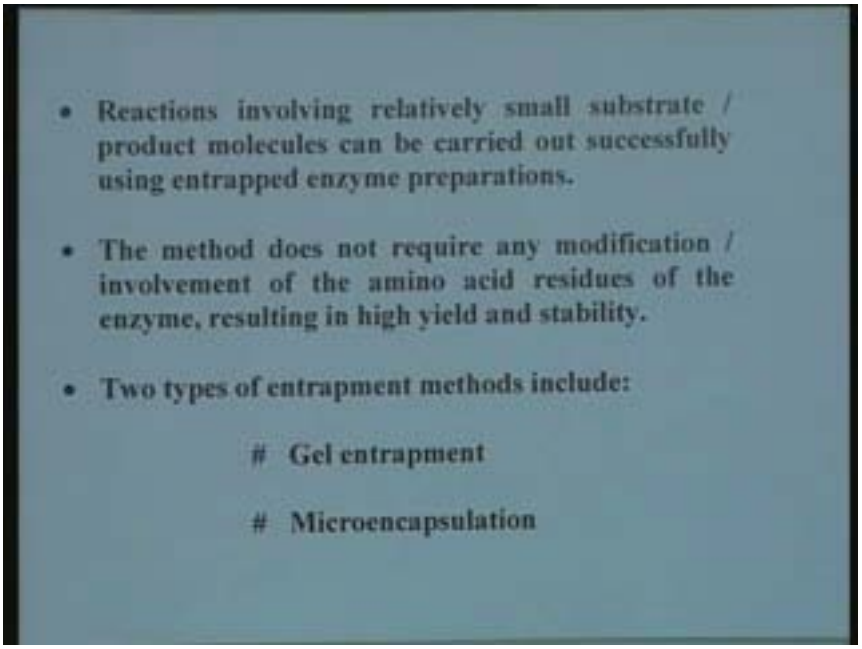

Because the enzyme is entrapped it does not have any interactions or modifications or involvement or coupling of the amino acid residues of the protein molecules with that of the matrix of the immobilize enzyme preparation and therefore the sole force that keeps the enzyme molecules bound to the matrix is the porous network and purely by molecular size. Therefore only small molecular weight substrate and products can be processed while the large molecular weight substrate or products cannot be processed using entrapped immobilized enzyme preparations. The major advantage of this method is that it does not require any modification or involvements of the amino acids residues of the enzyme which results in the high yield and stability because there is no modification of the enzyme structure involved in the entrapment methods. It is purely a physical restriction constraint of the enzyme molecule within a narrow region of a space and therefore the enzyme does not undergo any deactivation and usually the yields obtained by the method are quite high.

Second thing is that the micro environment of the enzyme can also be tailored to suite its stability. While immobilizing the preparation, one can add substrate molecules or other stabilizing agents so that they are retained along with the enzymes and stable preparations can be obtained. Two major types of entrapments methods that have been extensively

followed are by gel entrapment. That means you have a polymeric gel matrix and an enzyme molecule is entrapped within the lattices or interstitial spaces of these gel just by physical confinement.

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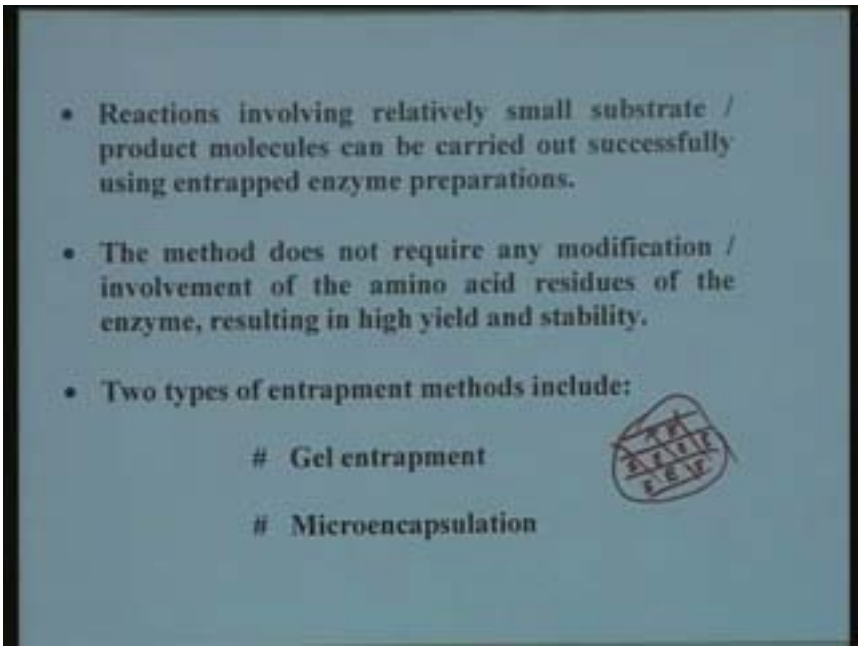

There are varieties of methods to make the gel network. We will discuss that and on the other hand the second method is microencapsulation where you tend to form a kind of a artificial cell with its boundary of a semi permeable membrane and the enzyme molecules are entrapped within this membrane and these micro capsules can be used as the immobilized enzyme preparations.

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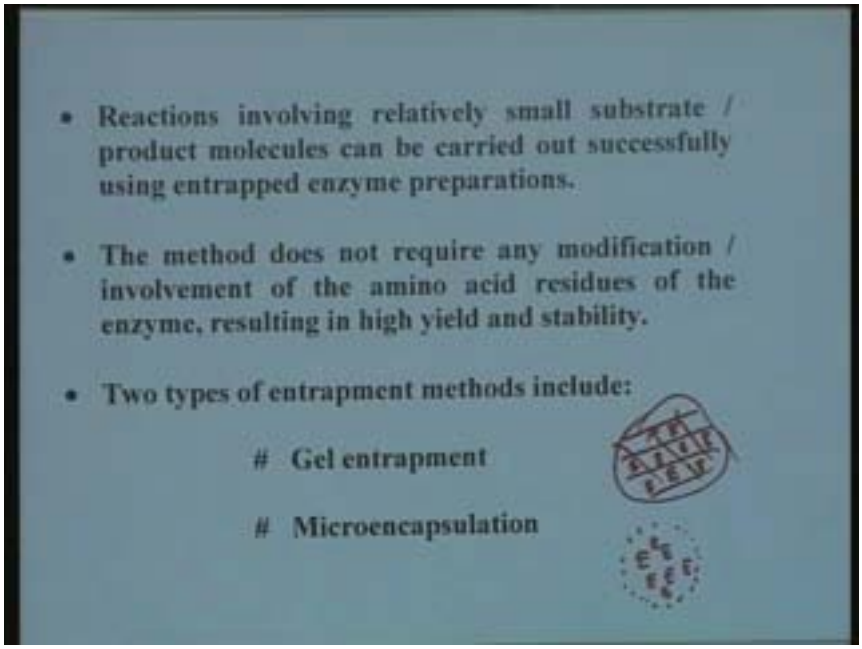

Looking at the first method that is the gel entrapment it involves entrapping the enzyme within the interstitial spaces of cross linked water soluble polymeric gels. Look at some of the phrases which I mentioned; cross linked water insoluble polymeric gels.

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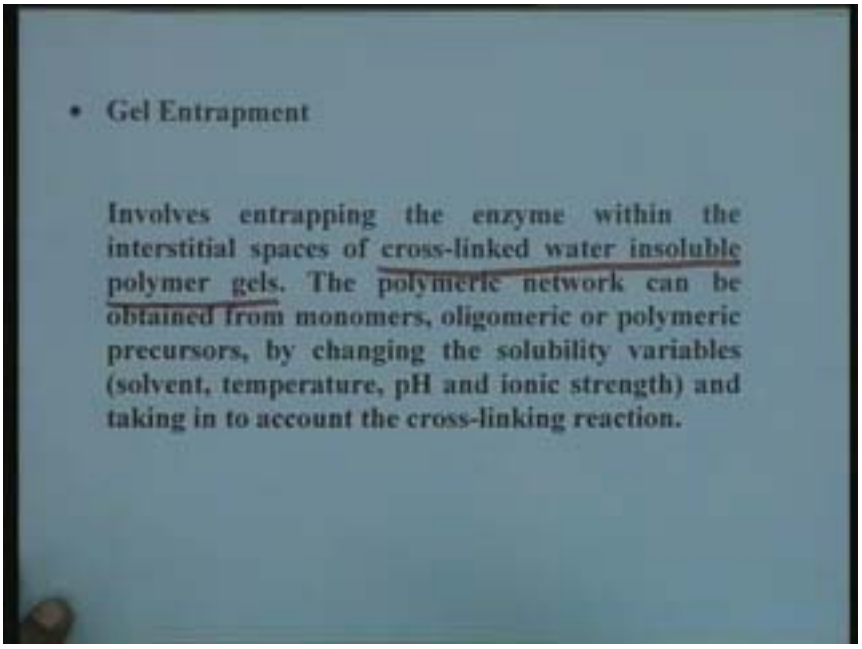

In most cases we start either with the monomer or a water soluble polymer. We start with a monomer unit and only those cases where the polymerization can be carried out under mild reaction conditions in the presence of enzyme. The monomer solution which is usually water soluble is mixed with the enzyme solution and conditions are created so

that polymerization can take place under mild reaction conditions with the help of a catalyst or initiator or some other conditions and the network of polymeric gel is formed which is water insoluble and then the enzyme is entrapped within the interstitial spaces. Or alternatively we take a water soluble polymer large molecular weight substance and mix the enzyme solution with this and the mixture is subjected to conditions by which the insolublisation takes place. Therefore it could be either by change of solvent, it could be change of by certain ions, it could be by temperature; the usual parameters which can induce insolubility or it can affect the solubility of the polymer. The polymeric network can be obtained from monomers, oligomers or polymeric precursors by changing the solubility variables. So there are two approaches by which these preparations are made by changing the solubility variables like solvent, temperature, pH and ionic strength or the cross linking reactions.

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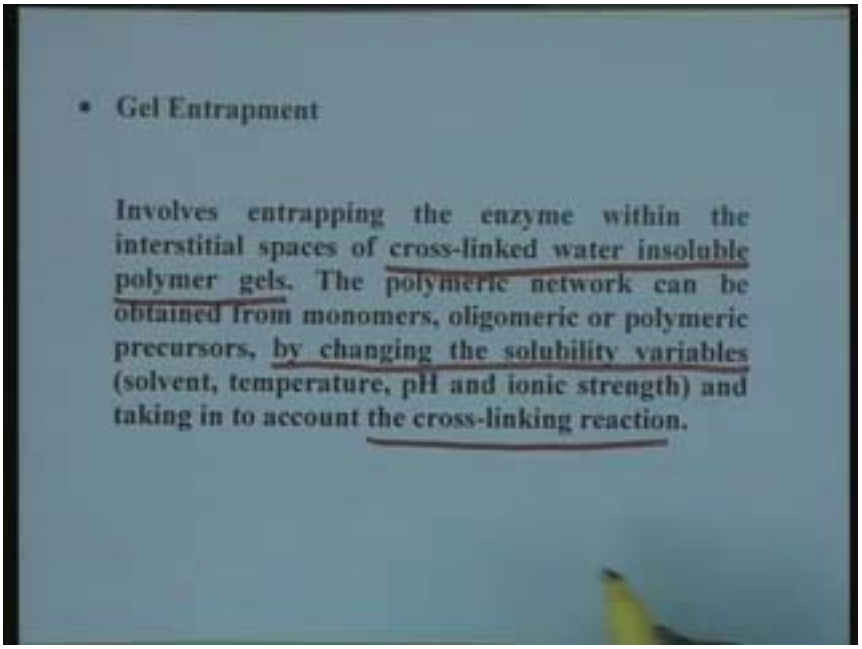

If it is a water soluble polymer, one can also induce cross linking by certain conditions so that it becomes water insoluble and the enzyme gets trapped into the matrix.

You see the purpose is if we start with water soluble polymer it will usually not have any cross linking. It will be water soluble with lot of hydrophilic groups and the enzyme can easily mix with the solution. Once you induce cross linking into it by any method, it could be by a cross linking bifunctional reagent like butyraldehyde, it could be ferric chloride. Variety of methods has been employed and after cross linking is induced the whole network becomes water insoluble and the enzyme molecules are entrapped. On the other hand when we are starting with a monomer unit which is water soluble, then to get water insoluble gel you include a cross linking agent. These two parameters are in ….. (9:31). One is the degree of cross linking and the other is to change the solubility parameter. The ultimate matrix must be insoluble in water and also it should be in a

proper range of cross linking such that the porosity can be regulated. Cross linking helps in a very significant manner to control the porosity of the matrix; higher the cross linking smaller is the porosity. One of the most commonly used methods is the poly acrylamide gel entrapment. This is poly acrylamide gel entrapment.

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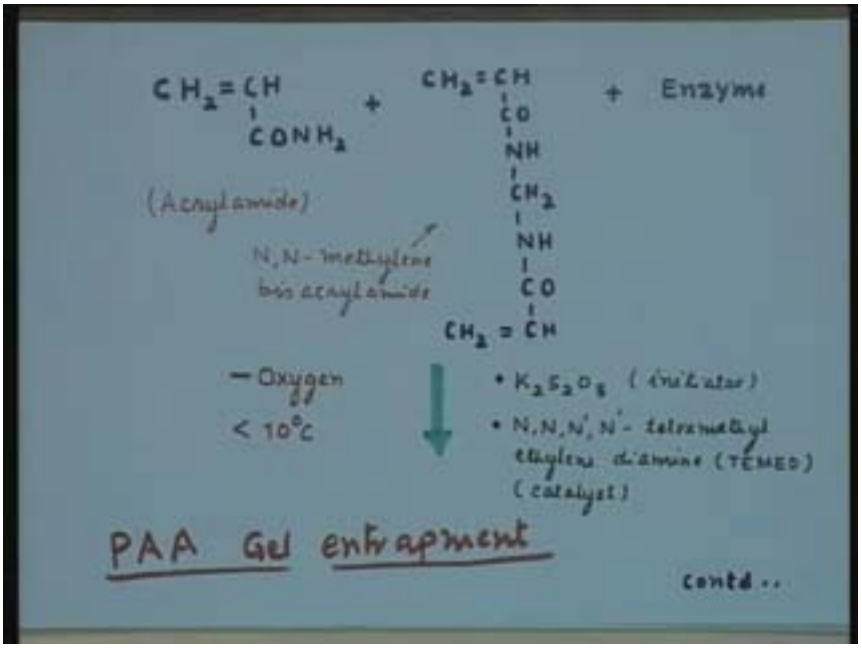

More than 50% of the total literature cited on entrapment methods would probably be devoted to polyacrylamide gel entrapment. It was one of the earliest methods also because the poly acrylamide gel preparation was an existing methodology even for gel electrophoresis. So we will use the same method to prepare an enzyme containing gels. The method is very simple. We start with an acrylamide monomer which is water soluble. To this a cross linking reagent usually N,N- methylene bisacrylamide usually often called as BIS is added and an enzyme solution is mixed with it. The three water soluble materials are mixed to which an initiator is added. Number of initiator molecules can be used. It could be ammonium persulphate or potassium per sulphate or riboflavin. They are initiators; that means it is a free radical polymerization. So you need an initiator molecule which can initiate the free radical reaction and TEMED, N,N,N',N' tetra methyl ethylene diamine is used as the catalyst. The two parameters which are very important to control here is the absence of oxygen. The presence of even trace of oxygen inhibits polymerization reaction. So it is very important that all the reagents that have been used are free of oxygen. If you bubble nitrogen through the reagents you can get them free of oxygen. But even traces of oxygen can reduce degree of polymerization in the polymerization reaction.

The second parameter is that the reaction must be carried out at less then ten degree centigrade. The reaction temperature while it will control the reaction rate it will carry out the reaction at a slow rate and it has also been seen in the polymerization reaction

that slower the rate degree of the polymerization can be taken up to a higher level. Secondly it is also important to keep the enzyme in a good state that is not to allow inactivation of the enzyme. The reaction is exothermic and lot of heat is generated. So you have to make arrangement for dissipation of heat so that the temperature can be maintained at less than ten degree centigrade and the enzyme is not deactivated. It's a very fast reaction. The whole reaction will be over in a couple of minutes. In a couple of minutes you get a polymeric network of chains. It's a three dimensional network. These are the chains which are shown in the dotted lines and indicate the cross linkages which are vertical to the plane of the paper

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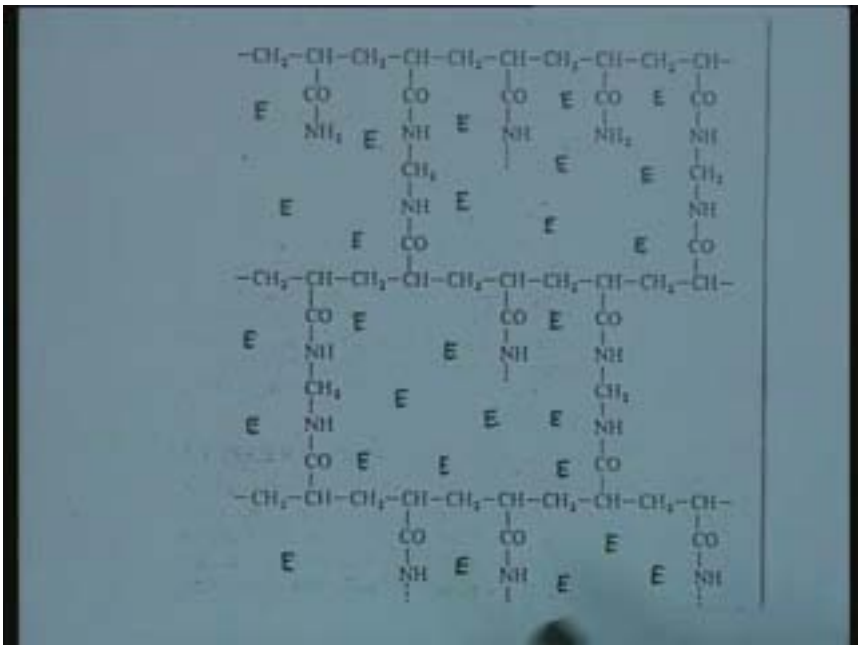

So the three dimensional network is formed and enzyme molecules are entrapped within the interstitial spaces which are created by the cross linkages that are maintained and you get a smooth solid block in the form of a gel. The porosity of the gel can be maintained by the concentration of the cross linking agent that is BIS. Temperature also can control the degree of polymerization and all the parameters can be optimized for a given condition so that the maximum yield of the enzyme activity is recovered at the end of the reaction.

You end up in a three dimensional network which is water insoluble and in the form of a block. Here some of the cross linkages are shown ending with the dots. These ones are perpendicular to the plane of paper to indicate a three dimensional network and the enzyme being entrapped within the space. The conditions of concentration of the acrylamide monomer, the concentration of the cross linking agent, that is BIS, the reaction temperature are the three major parameters which control the degree of polymerization and porosity of the gel. After the gel is formed, the gel is mechanically

ruptured into small pieces in the form of a small granules or cut into small pieces and then can be used as a packing material in the reactors.

In addition to this another very common group of methods which has been used are the use of photocrosslinkable reagent prepolymers. These are mostly oligomers which can be polymerized using UV light.

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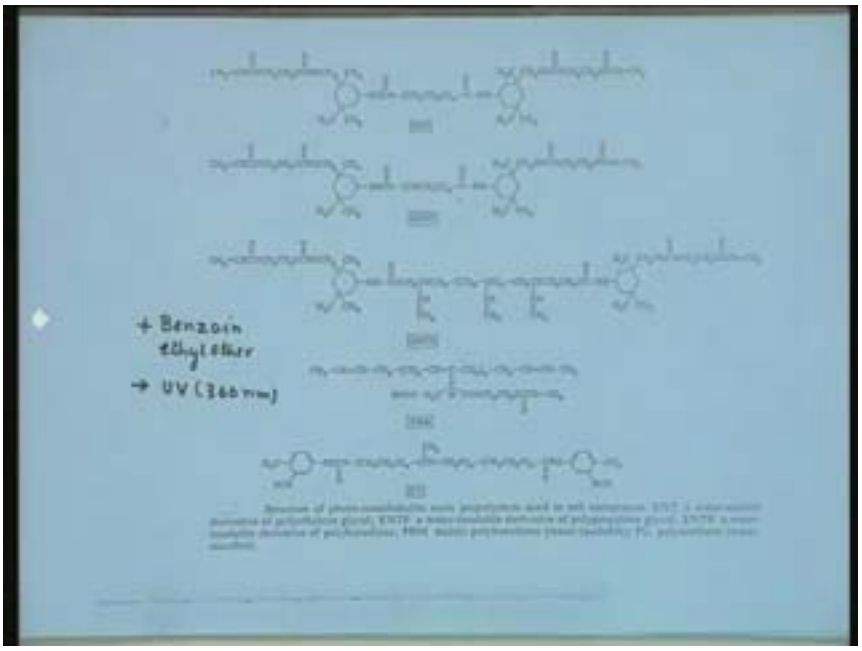

A large number of methods are based on the use of photocrosslinkable prepolymers. I have shown here some of the reagents like ENT which is water soluble derivative of poly ethylene glycol, ENTP is a water insoluble derivative of polypropylene glycol and ENTB is a water insoluble derivative of polybutadiene. PBM is malic polybutadiene and PU is polyurethane and these are the precursors of certain photocrosslinkable polymers. These molecules can be polymerized using UV radiations in the presence of a photoinducer, a catalyst, and benzoin ethylether is used in most cases as an inducer for photocrosslinking. These materials can be dissolved along with the enzyme and then polymerization can be started by exposing them to ultra violet light at 360nm for few minutes and then in few minutes the reaction is over and the gel preparation containing the enzyme molecule is made available.

Another group of entrapment methods, gel entrapment method rather, is from the long chain polymers. There are two major approaches by which these gel entrapment preparations are made from long chain pre formed polymers.

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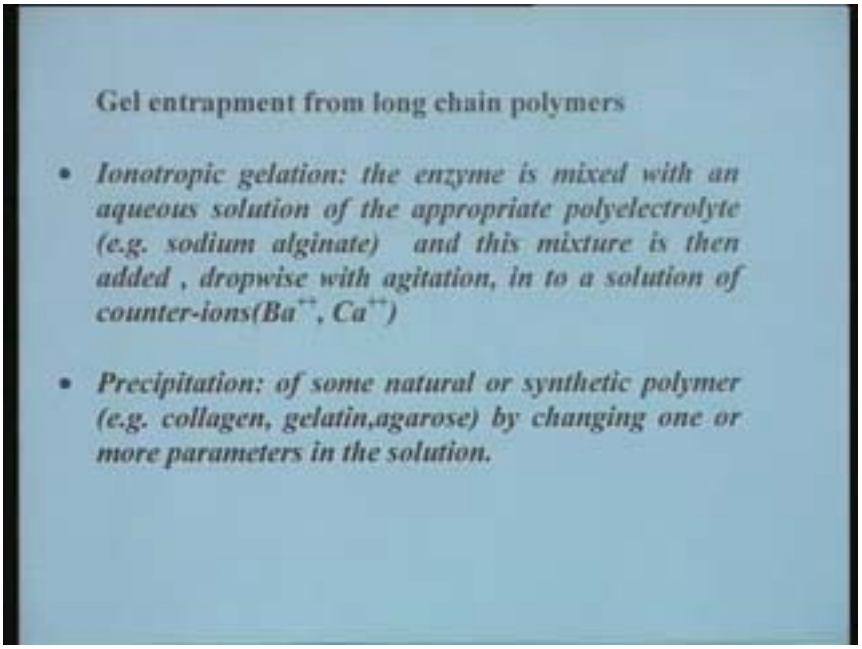

In the two earlier methods we have talked about the use of a monomer and an oligomer a prepolymer. On the other hand here we are using a ready made polymer. The first method which is also a very commonly used method is ionotropic gelation in which the whole process of insolubilization of the polymer is based on ionic exchange. The enzyme, water soluble enzyme is mixed with an aqueous solution of the appropriate polyelectrolyte. Most of the polymers used here are polyelectrolytes which can be mixed with the enzyme solution and a typical preparation which has been reported is sodium alginate. It is a polysaccharide, negatively charged polyelectrolyte. This polyelectrolyte is then mixed with the enzyme solution and this mixture is then added drop wise with addition into a solution of counter ions. The sodium is replaced by calcium. Calcium has been most commonly used so instead of sodium alginate you end up in calcium alginate which is water insoluble.

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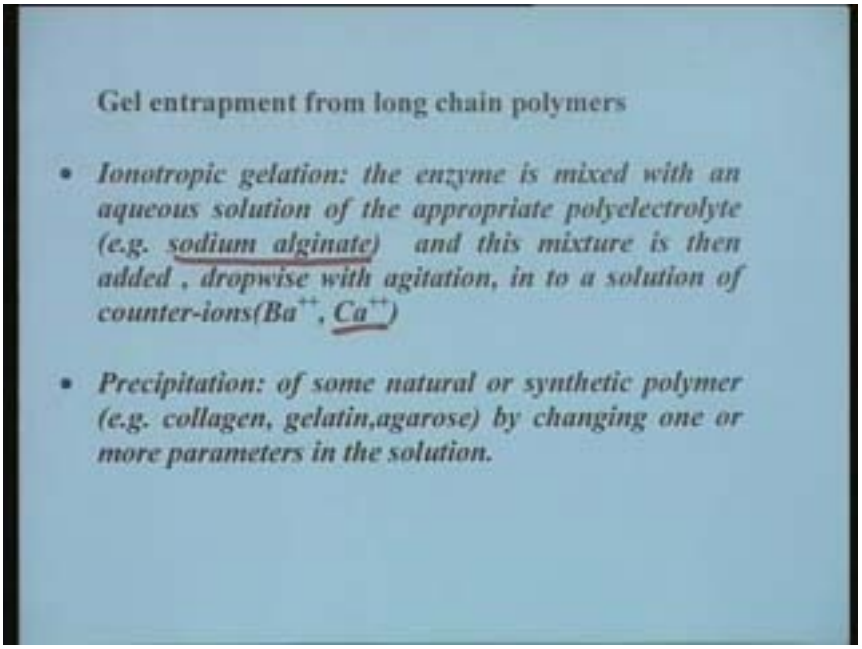

While sodium alginate is water soluble polymer, it can mix with the enzyme solution and then this mixture is extruded through a fine syringe into a calcium chloride solution and agitated. By the exchange of ions the sodium gets exchanged with the calcium, you get water insoluble beads. One of the major advantages of this method is that while in the case of previous methods you get the block of the gel and the gel block has to be mechanically dispersed into a particular shape for use in a reactor, here you can prepare the beads, the immobilized enzyme preparation, almost in the bead form so that that can be directly used. One of the disadvantages here is the stability of this material. For example calcium alginate although insoluble in aqueous medium gets solubilised by certain ions like phosphate ions. If your medium contains phosphate ions, calcium alginate will slowly get dislodged and you will end up in dissolution of immobilized preparation and sometimes instead of calcium, barium or aluminium is used as a counter ion. Such methods are very commonly used not only for enzymes but even for some of other biocatalysts like …… (19.58) cells, this provides a very viable method for immobilization.

Second method is based on precipitation. That means you change certain conditions in a way that the mixture of the enzyme and the prepolymer changes the solubility behavior and gets precipitated.

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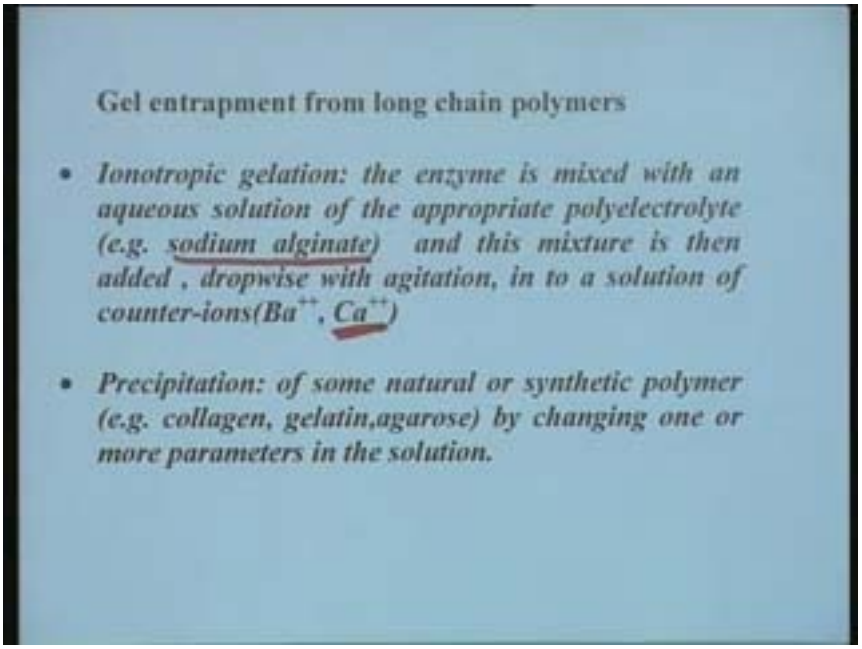

Some of the natural synthetic polymers that are very commonly used are: collagen, gelatin and agarose. Collagen and gelatin are proteins whereas agarose is a polysaccharide. A large variety of natural polymers are used by changing one or more of the parameters in the solution. Collagen which is a structure of protein, is found in the slaughter house waste. Collagen can be taken mixed with the enzyme solution and you make a dispersion of the enzyme with the collagen solution. You don't really get a good solution. Being a structural protein, you get a real dispersion of the enzyme. This dispersion is then cast on a sheet so that you can get thin film of the enzyme entrapped in the collagen membrane. To provide stability to this film usually this film is subjected to crosslinking either by butyraldehyde or by ferric chloride. Different methods have been used; so crosslinking becomes an important parameter to provide stability to the immobilized preparation. Similarly in the case of gelatin, one can mix the gelatin solution with the enzyme solution and pull it. Pulling will reduce the solubility and we will get a gel. After the gel is formed then you can subject it to butyraldehyde to provide crosslinking and therefore get a permanently insoluble gel preparation. The same applies to agarose which is also a polysaccharide and also can be converted into a gel by cooling and there are some other polymers including synthetic polymers which have been used by this mode into immobilized preparation.

Collagen is not so good. It forms the dispersion with the enzyme molecule and a collagen method is not exactly like a gel as we consider a poly acrylamide gel or an agarose gel or any starch gel. It is a dispersion which is cast into a thin film. Dispersion of collagen and enzyme in water is spread over a film, thin film usually. Preparation like this are made in the form of thin films and then to give the stability they are crosslinked and after it is crosslinked it becomes a permanent. You just add the cross linking agent after a film has been made. Yes algenic acid.

Another method, a very useful method, again for immobilizing mostly microbial cells is fiber entrapment.

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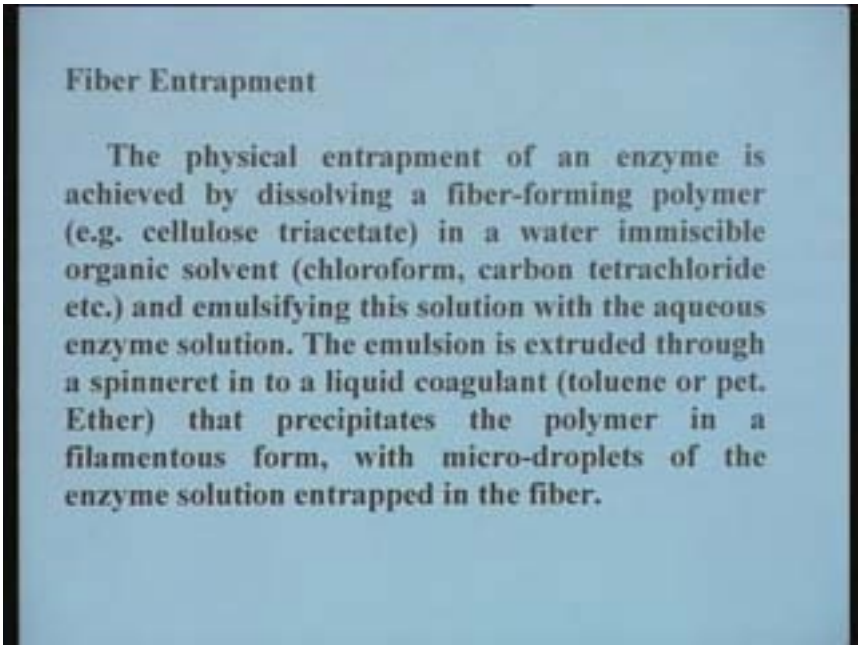

The physical entrapment of an enzyme is achieved by dissolving a fiber forming polymer like cellulose triacetate which is water insoluble. This is dissolved in a non-polar solvent like chloroform or carbon tetrachloride, methylenechloride and this organic solution of cellulose triacetate is then emulsified with the aqueous enzyme solution. Stir the two solutions inorganic as well as the aqueous phase; agitate it and you get a fine emulsion. This time emulsion is extruded through a spinneret just like you make nylon fibers and pass through a fine spinneret and the extruded fibers or the filaments are then dipped into a liquid coagulant like toluene or petroleum ether. When these fibers are passed through toluene, they get coagulated, the polymer gets coagulated with the water droplets, the aqueous droplet of enzyme being entrapped in the fiber.

This is a spinneret. This is put into a trough containing toluene. When it passes through toluene after residence time in toluene, the polymer cellulose triacetate gets coagulated; it is no longer soluble and then the fiber that are formed contain the enzyme micro-droplets which was earlier emulsified into the solution of cellulose triacetate which are entrapped in the fibers.

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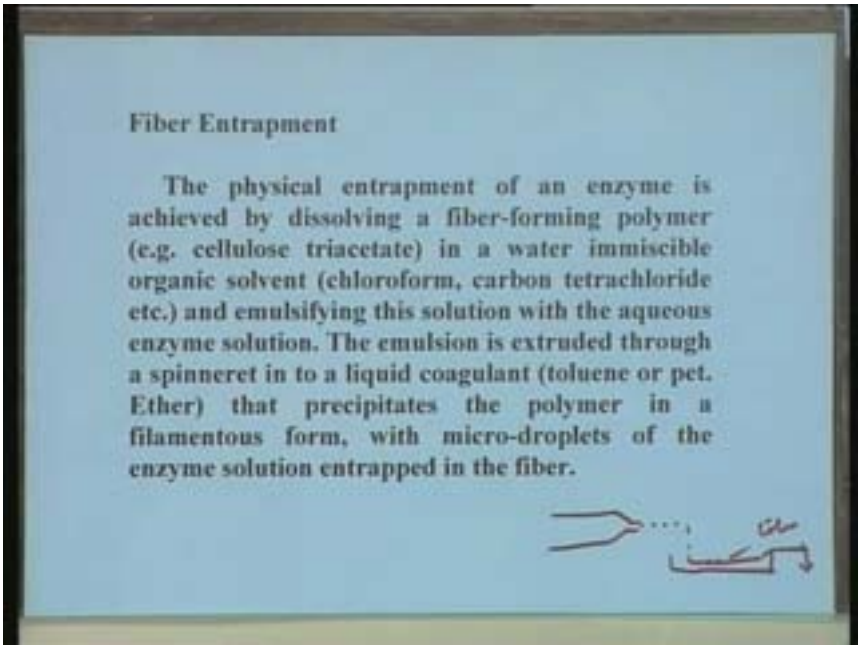

Then these fibers can be used in a variety of modes in a reactor. Even people have tried to make fabric out of immobilized cells which then can be used for variety of industrial applications.

The second group of entrapment method is on microencapsulation and in the case of micro encapsulation, as I mentioned earlier, the enzyme is enclosed within a semi permeable membrane; polymeric membrane permeable to substrate and product. Enzyme does not pass through but substrate or product must be able to pass through the membrane in the system.

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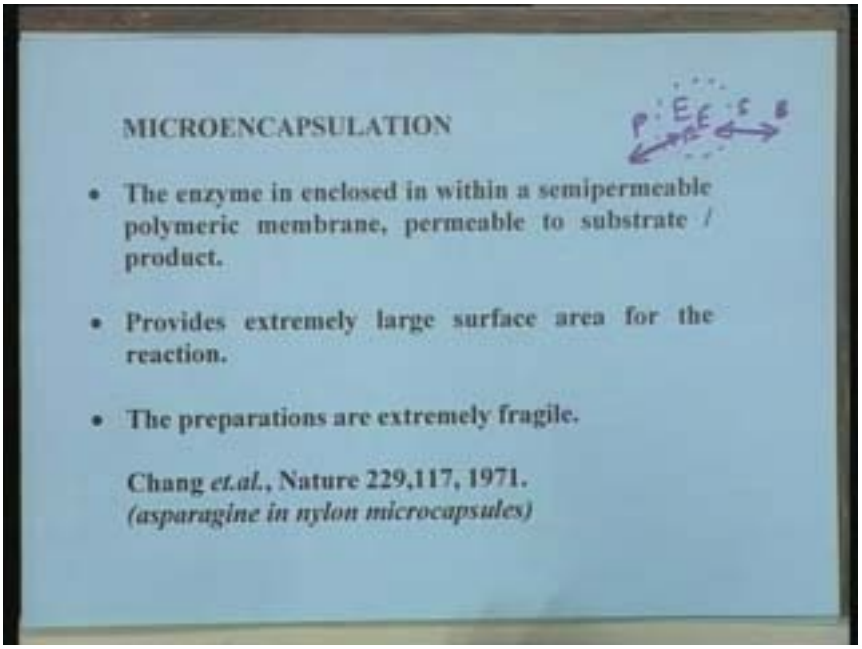

One of the major advantages is that it provides extremely large surface area for the enzyme substrate complex and the enzyme substrate contact within a small volume. But the preparations are too fragile because this semipermeable membrane is very, very thin and it provides you a very, very fragile preparation. Therefore its use in a number of industrial reactors is probably very difficult because it will not be able to withstand the shear and the mechanical load which an industrial reactor for chemical processing will require. But this method has been very extensively and very favorably used particularly for biomedical applications. For example the references as I have listed here by Chand and associates, which was reported in 1971 had asparagine in nylon microcapsules. In the preparation of nylon microcapsules, an aqueous solution of the enzyme and hydrophilic monomer like hexamethylene diamine is dissolved in a buffer. This is emulsified in a water immiscible organic solvent which is cyclohexane, chloroform mixture. To the emulsified monomer solution, you add another hydrophobic monomer dissolved in the same solvent. Hydrophobic monomer, in this case is sebacoyl chloride. At the interface the two monomers gets polymerized and the reaction gives you a polymer what we know as nylon. So the chain continues and you get nylon. Particularly the enzyme which was mixed with the hydrophilic monomer is entrapped within the microcapsule.

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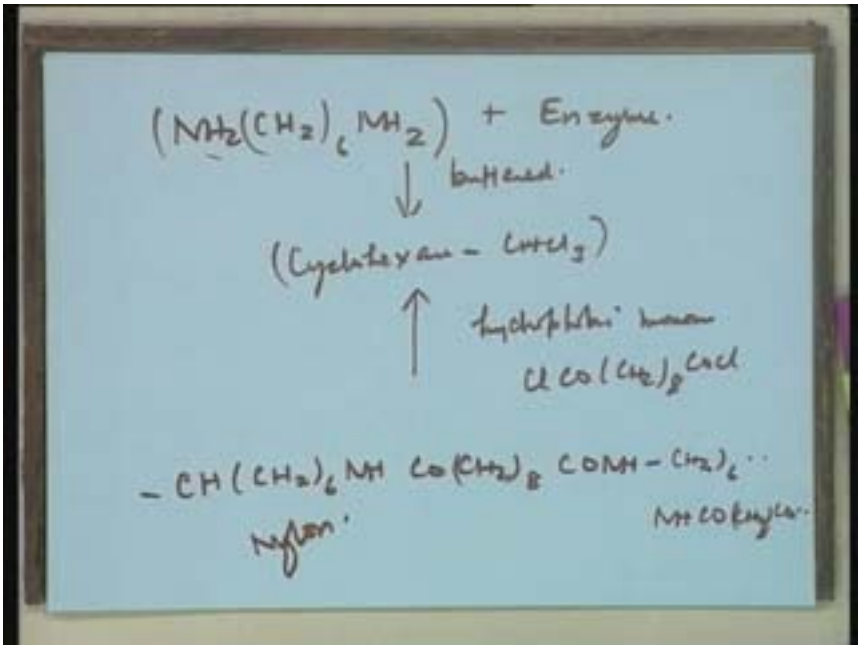

This method has been used very significantly for immobilizing an enzyme called asparaginase which is used as a therapeutic agent for persons suffering from leukemia. One major advantage here is that the nylon molecule being non-immunogenic provides a much better material for putting into the human system. Carbohydrates or proteins are known to generate immunogenic reactions in the body whereas nylon is relatively inert as far as the immunogenecity is concerned and these capsules have been used for treatment of leukemia. For most of the patients suffering from leukemia one of the problems is their lack of asparagine. They lose the asparaginase enzyme, the enzyme which hydrolyses the asparagine in the system. While these methods have academic significance from the point of view of providing certain characteristics features but for industrial reactors they are not very suitable. I found applications in only biomedical applications.

Right. What you have done is initially you have taken a hydrophilic monomer and the enzyme solution in a buffer, in an aqueous buffer, mixed it into an organic solvent. So you make a fine droplet, fine emulsion. To this emulsion we add the hydrophobic monomer dissolved in the same solvent. So at the interface of the hydrophilic and hydrophobic monomer they polymerize and the enzyme gets entrapped into and these microcapsules are very fine in size. The size can be varied from almost about two to three microns to ten microns almost like an artificial cell, a yeast cell for example. Almost they will be the size of yeast cells.

We have seen so far a large variety of immobilization methods. If one has to really study various methods I think one must compare the various methods and to evaluate various methods for a particular job then these are the parameters which are needed to be looked into. On the right hand side I have given you the various methods that we have talked about. On the left hand side are the parameters which are important to be compared and there is no method absolutely reported in literature which can meet all the requirements.

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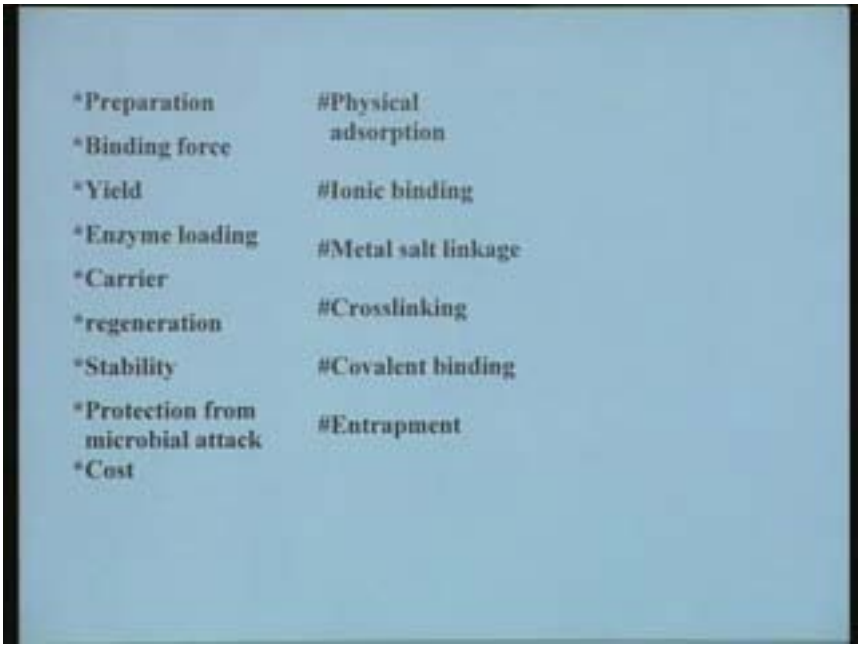

There is no ideal immobilization method and each method has to be weighed in terms of its applicability and the parameters that are important are: method of preparation, binding force for example I have mentioned adsorption as a weak binding force as compared to covalent binding. If you require a very strong binding force use covalent coupling. Yield is a very important parameter. Enzyme loading …. (33:26) per unit carrier how much of the enzyme can be loaded and it is something like specific activity of a soluble enzyme preparation. That means in so much of protein what activity can be carried out? Similarly enzyme loading in terms of carrier loading. Then the choice of carrier and in the choice of carrier the regeneration of the carrier is also associated. The cost of the carrier and the regeneration capacity are directly linked. The stability of the preparation and protection from microbial attack. Most of the entrapment methods have a major advantage of protection from microbial attack because they are constrained within the four walls of a structure almost like microbial cells where all the enzymes or proteins are enclosed within a cell wall in the membrane and within a living cell the enzyme remains safe from microbial attack. Similar situations can be looked from entrapment methods and finally the over riding factor often comes as cost. So all the methods are mostly considered in terms of these parameters.

When we talk of the immobilization for industrial reactors the major factor is the cost and in terms of the cost it is desirable that when we produce them on a commercial scale using microbial cells, those enzymes which are intercellular in nature they could be used almost in the form of ….. (35:18). You need not disrupt the cells and then isolate and purify the enzyme and then immobilize.

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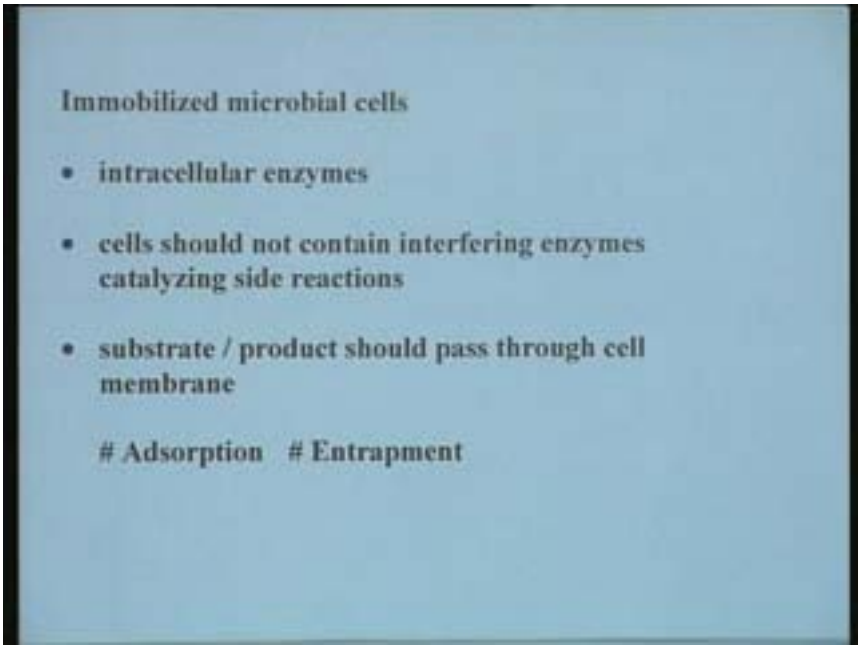

Because isolation, purification is a very cost intensive operation for intercellular enzymes. So in many cases of intercellular enzymes efforts have been made to immobilize the whole cell itself and the immobilization of the whole cell is a desirable feature particularly in the case of intercellular enzymes. A major application of immobilized cells has been in isomerisation of glucose to fructose and the process is only techno economically viable because it can be used in the form of immobilized cells. If the enzyme, the glucose isomerase is an intercellular enzyme and if one has to really isolate, purify and then immobilize probably the process will not be viable. Today the process is used industrially, commercially almost in half of the world wherever sucrose is not abundantly available, as a sweetener for glucose fructose mixture because the product cost is not very high. Therefore the use of immobilized cells provides a very viable alternative.

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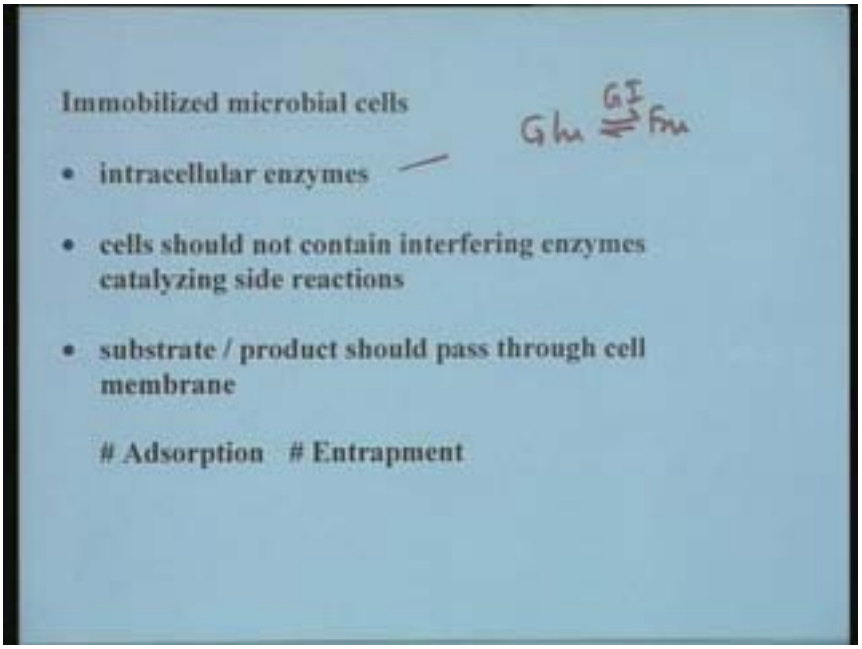

The conditions that are imposed on the use of immobilized cells are that the cells should not contain the interfering enzymes catalyzing the side reactions. That means for example if you are using glucose as a substrate for a particular reaction, then the cell should not contain the enzymes that can also act on glucose and produce the side effects. Glucose is a substrate. You will all agree that it is very difficult to look at the living cell or a microbial cell which will not have more than one enzyme and which will use glucose as substrate. As in the very first reaction itself for example hexokinase will act on glucose as a substrate and produce the corresponding phosphorylated sugar. So in that case the other alternative is that it should be feasible that other interfering enzyme if there are any it should be possible to inactivate them by some pretreatment which does not influence the desired enzyme.

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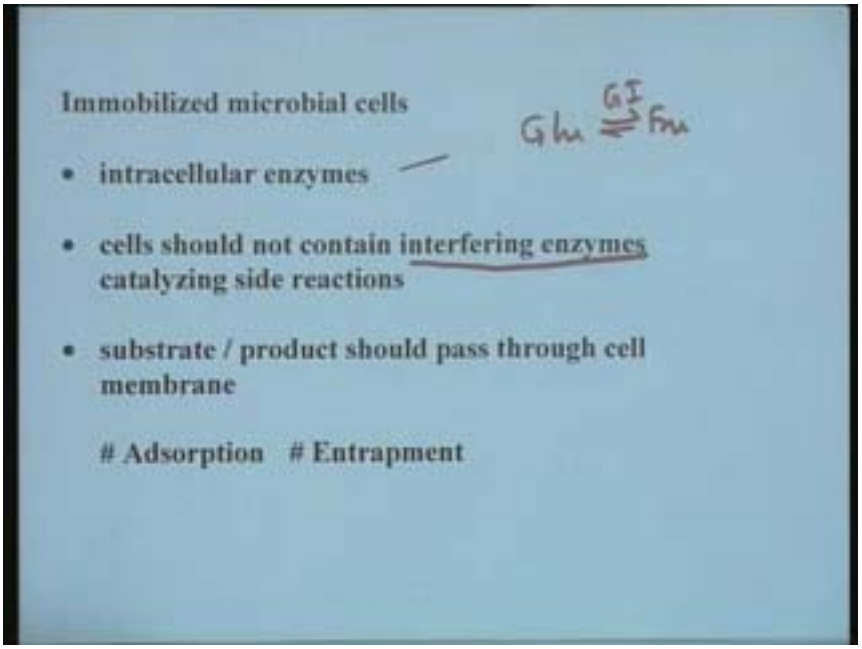

In the case of glucose isomerase or the immobilized glucose isomer is the form of …. (37:46) we take advantage of the thermal stability of the enzyme. The enzyme glucose isomerase is a very thermo tolerant enzyme of the order that the optimum temperature of the glucose isomer ranges between 70 to 80^{o}c and therefore you can take the cells give a heat treatment whereby most of the other enzymes which are able to act on glucose are inactivated but glucose isomerase still remains active and use that heat treated cells for immobilization. So the cell should not contain any interfering enzyme. There are many preparations like for example preparation of fumaric acid from aspartic acid where the enzymes are too specific and usually there are no other enzymes present in the cell which will be able to utilize those substrate. Then the substrate product should also pass through the cell membrane. That is the major constraint because the enzyme is located within the cell. So the cell membrane should be able to transport the substrate and the product across the boundary otherwise you cannot make the reaction feasible.

For immobilized cell although theoretically almost all methods, variety of methods starting from adsorption to entrapment have been used sparingly, the bulk of the methods that had been used rather ….. (39:14) on industrial scale are based on adsorption or entrapment. Most of the entrapment methods very commonly have been used to entrap the microbial cells and as you will notice that entrapment within calcium alginate beads, polyacrylamide gel and cellulose acetate fibers have been commonly used methods for immobilizing. The method remains the same as we have talked about the enzyme molecules. The entrapment of cells is much more because of their larger size than the enzyme molecule. Their retention in the entrapped method is far superior compared to the soluble enzyme. There are four adsorption methods which are based mostly on ionic binding because most of the cells have negative charge on the surface and therefore any positively charged matrix can be used to adsorb the cells on to the surface. These adsorb preparations are usually in dynamic equilibrium. That means if it is a growing cell then part of the cell will attach and some of the cells will be leaking out also. But in the case

of non-viable cells usually entrapment methods are the method of choice. We must here also probably make a distinction between living and non-viable cells.

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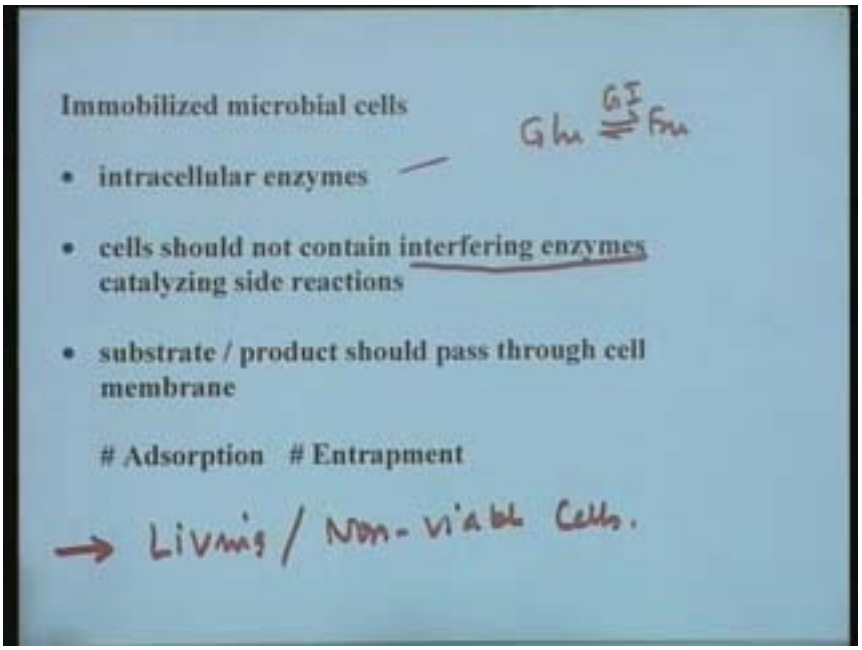

Living cells are usually used in those cases of bio-conversion processes where we require a series of enzyme reaction. More than one enzyme reactions is involved usually with a co factor regeneration that means a metabolic path way is involved with a metabolite to be produced or else if a single enzyme is desirable then it is always desirable to use non-viable cells. For living cells usually adsorption has been a method which had been very commonly used while for non-viable cells, entrapment is the method which has been most widely used. But the technical details of methodology for immobilization remain the same as in the case of enzymes.

When we talk of immobilization of microbial cells we could think of the cells which are viable, which can multiply. These multiplying cells or living cells are needed in those cases where the process requires a full metabolic path way which means a series of enzyme reactions which are interlinked for co-factor regeneration or for some other metabolite I mean uptake and production. In those cases living cells are used and usually these living cells have been immobilized by adsorption method because when they are living on the adsorption method although it is a method which regularly leaks out the cells from surface but then the new cells which are growing during growth phase they can also bind to the surface and then …..(42:43) in a dynamic balance. On the other hand if a single enzyme catalyzed reaction is the desired feature, then non-viable cells are desirable and they can be immobilized safely by one of the entrapment methods that we have talked about.

Having looked into a variety of immobilization methods, we should also have a code to characterize an immobilized enzyme preparation by any method.

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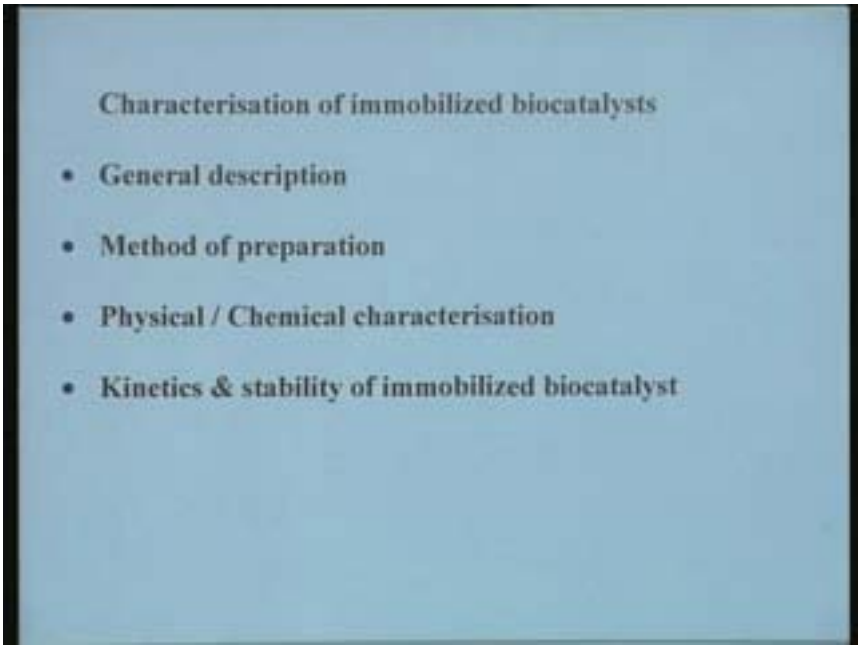

Somebody has reported an immobilization method for a particular biocatalyst. There has to be a common feature by which the immobilized preparation can be characterized so that the purpose of characterization is that the method should be reproducible by anybody. Minimum information should be provided so that anybody who wants to reproduce the method can use it for further work. Some of these steps which I have listed here provide this. For example when we say general description I am talking of the reaction scheme that is the reaction which you are looking at catalyzing; then the source of enzyme - which enzyme and from what source has been used; carrier. These three things must be clearly outlined. What is the reaction we are looking at? That means also involves which enzyme we want and what are the source of enzyme and the carrier which is required to be used for any method. The second step is method of immobilization. That means how do you prepare; what are the reaction conditions? That means the reaction conditions and particularly more important is yield, loss of activity and the activity left in the supernatent. So all that information must be provided in the methods of preparation. Then the characteristics feature is physical-chemical characterization which means the biocatalyst shape, mean particle diameter or wet particle diameter. That means after swelling what is the swelling behavior of the particle and the compression behavior in the columns and abrasion. If it is to be required to be used in the ……. reactor the minimum ….. velocity and all those properties must be identified for a particular immobilized enzyme preparation and then we report any method.

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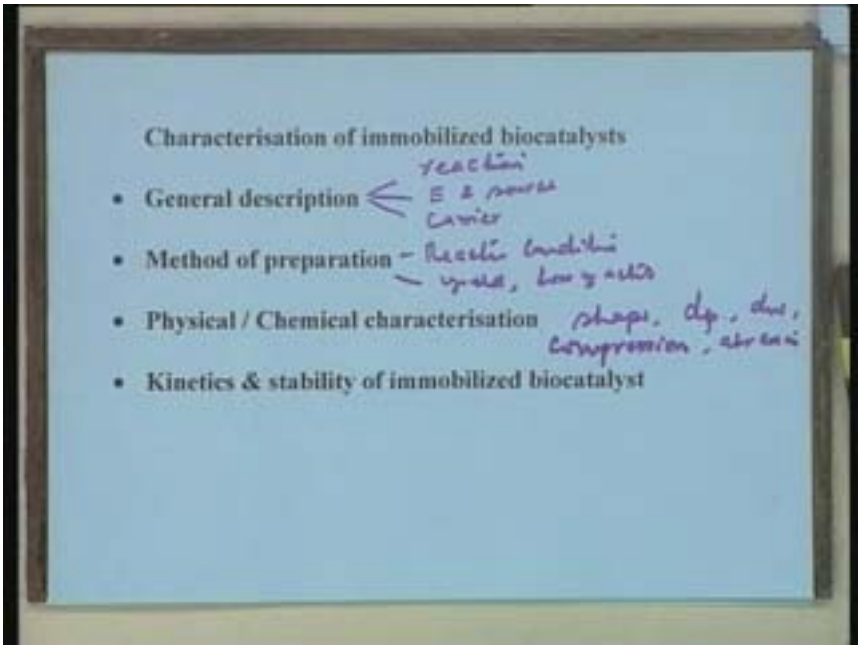

Finally kinetics and stability of immobilized biocatalyst a very important feature which probably dictates the choice of the immobilized enzyme preparation. As far as kinetics is concerned, as we have seen for the soluble enzyme, the substrate concentration versus initial reaction velocity that means the effect of substrate concentration on initial reaction velocity was the major kinetic characterization of soluble enzyme which gives you information about k_m, V_m and other kinetic parameters and normally by convention we represent the kinetic parameter for an immobilized enzyme or immobilized biocatalyst by k'_m and V'_m. Prime is added just to indicate that we are reporting an immobilized preparation, just as a convention. That information must be known and the kinetic characterization will imply that the k_m and V_m values for an immobilized enzyme preparation might undergo change during immobilization. Other than the reaction kinetics there are other physical parameters which get involved particularly diffusion and partitioning, which we will discuss in detail. Diffusional effects, then degree of conversion versus residence time at different initial substrate concentration, the reactor performance profile and finally the operational and storage stability. Data on all these parameters must be provided to characterize the particular immobilized enzyme preparation. We have so far discussed the first three parameters we will discuss this in our future discussion on the kinetics of immobilized enzyme.

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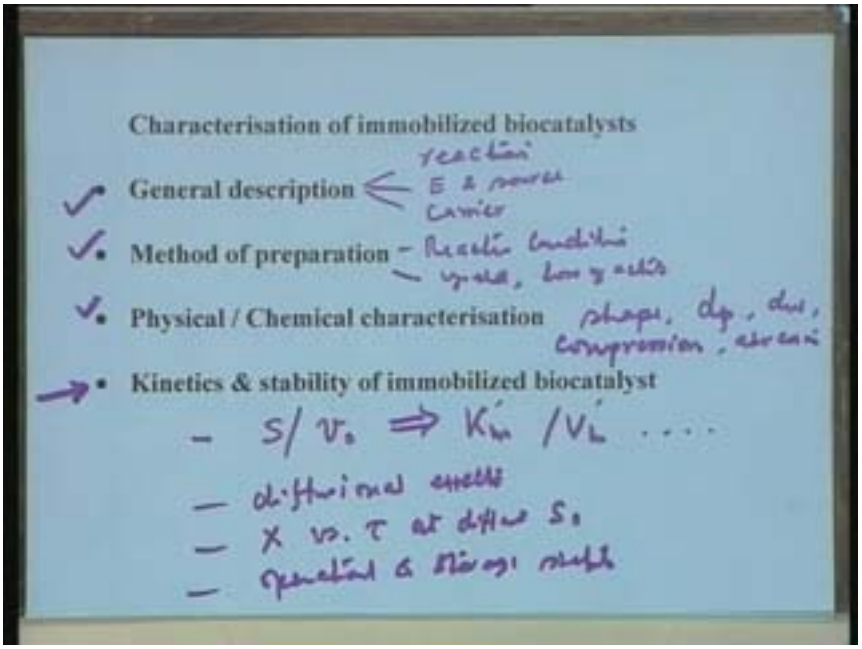

So we stop with this.