ENZYME SCIENCE AND ENGINEERING

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LECTURE–28

ENZYME TECHNOLOGY CHALLENGES AND FUTURE DIRECTIONS

Today we shall discuss about some of the issues and the future directions of enzyme technology that we are likely to face in near future. So far we have been talking about the status of enzyme technology as is being practiced in the industries as for the application of enzymes are concerned in different sectors of their application. The issues that I will be talking today are ones which are still posing challenge and are restricted to laboratory investigations so far and it is likely that some of them in a very short time will come out as industrial processing concepts.

First issue that I want to draw your attention is the bio processing with co-factor linked enzymes.

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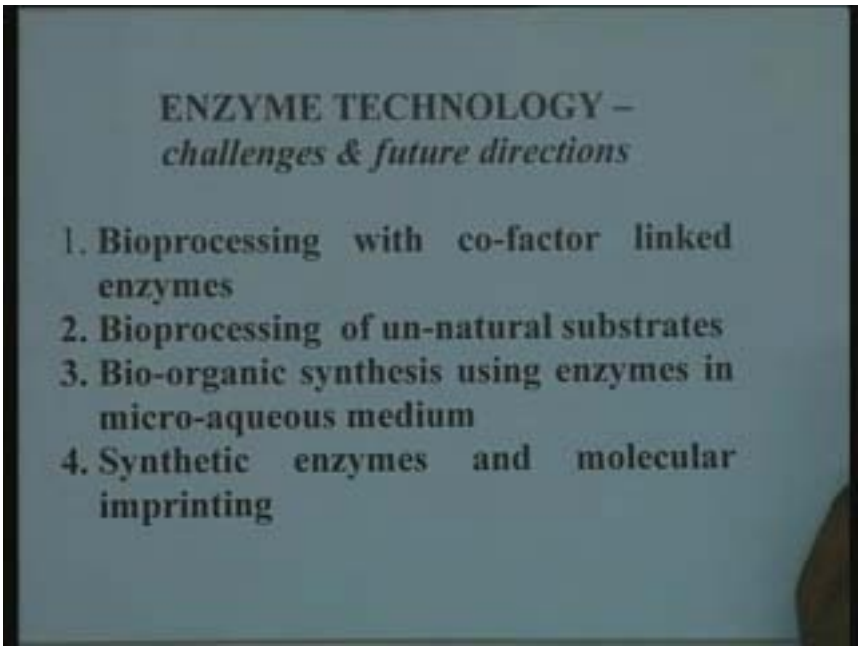

As I mentioned earlier that a large number of enzymes that are known are mediated through co-enzymes or co-factors as you know and most of the applications that we talked earlier, they related to only hydrolytic enzymes which do not require co-factors

with the sole exception of glucose isomerase which is an isomerase and it also does not require co-factor. The large number of cofactor linked enzymes therefore opens a big challenge to the industry or to the scientist who are concerned with the diversification of application of enzymes. The basic motivation or rational for the use of co-factor linked enzymes comes from the function of living cell in which a number of co-factor linked enzymes are operational in various metabolic path ways which are used by cells for carrying out their various catabolic as well as anabolic reactions.

The basic concept in a living cell, when it comes to cofactor linked enzymes, relates to cycling of the cofactor between two enzyme catalyzed reactions. The two enzyme catalyzed reactions are coupled in a cell in such a way that the cofactor which is used in one direction is recycled back through another reaction with which it is linked and the total equilibrium is in favor of the desired intermediate the direct part to be synthesized by the cell.

As far as their industrial application is concerned the issue is restricted to mainly three issues: one is that the coenzyme and the enzyme must be retained in the reactor. We have seen in our earlier deliberations where we have talked about immobilized enzymes that the enzyme can be retained in the reactor and used over a long period of time. A similar concept has to be developed for co-enzyme retention in the reactor. Although it is little more difficult in the sense that while enzymes provide you a large molecular size as well as a number of functional groups on the molecule which can be used for coupling to matrix where the required enzyme is a small molecular weight compound not such a large molecule and also the number of functional groups available on the molecule are very restricted. So exactly the same concept may not be applied, but still one needs to retain the co-enzyme so that it is not consumed during the reaction. The basic concept to retain the enzyme as listed here is the use of the water soluble polymer bound coenzyme. For retention of the coenzyme we use instead of a small molecular weight coenzyme, a water soluble polymer bound coenzyme.

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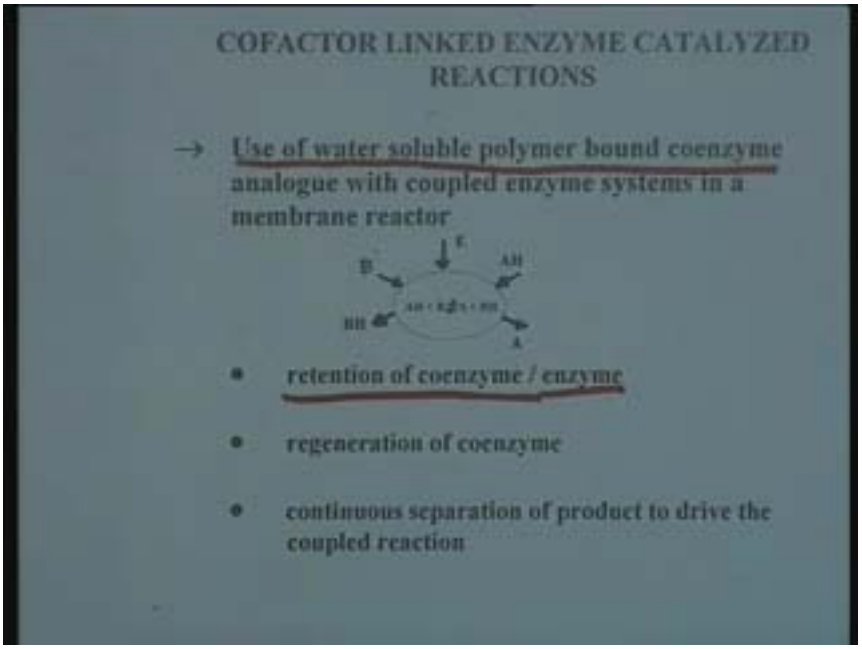

Because of binding purposes we add another functional group which can be used for coupling to a water soluble polymer which may not be present on the original coenzyme as it is and therefore you need an analogue. When I say analogue I am referring to some chemical and structural modifications but the role of coenzyme is retained by the molecule. The second issue is regeneration of the coenzyme. The regeneration of the co-enzyme like in a living cell can be carried out by coupling to enzyme catalyzed reaction, as you see here. In a membrane reactor in which a reaction has to be carried out, I illustrate a general redox reaction, AH plus B going to A plus BH. There is an oxidation of AH and reduction of B and this can be done by two enzymes. One of them oxidizes AH and produces A, where as the other one reduces B using the same co-factor in the reverse direction. If this co-enzyme is NAD, this has to be coupled to a polymer so that the coenzyme is not lost during use.

Another important feature is that the product needs to be separated to drive the coupled reaction and the separation of product is mediated by use of membrane reactor in which the high molecular weight enzyme as well as the coenzyme, which is now made to be high molecular weight, is retained in the reactor and thereby the product can be separated from the reaction mixture and the reaction can be driven in the desired direction.

I will illustrate the point with one of the examples. There is lot of chemistry involved because different people have proposed different kind of chemical reactions. I am not interested to give you detailed chemistry. Just to explain the concept for example you take polyacrylamide, which is a water soluble polymer. This is derivatised by hydrogen hydrate to polyacrylamide hydrazide and this polyacrylamide hydrazide then is coupled to a co-enzyme NAD which is derivatised in the form of carboxymethyl NAD. So therefore a carboxymethyl group is generated here and carboxymethyl NAD is then activated by carbodiimide, the reaction which you are familiar, with the carboxylic group containing enzymes by activation by carbodiimide and therefore you get an activated co-

enzyme molecule. A carboxymethyl which is NAD analogue is activated using carbodiimide under acidic conditions to give you an activated NAD. You can call it NAD activated.

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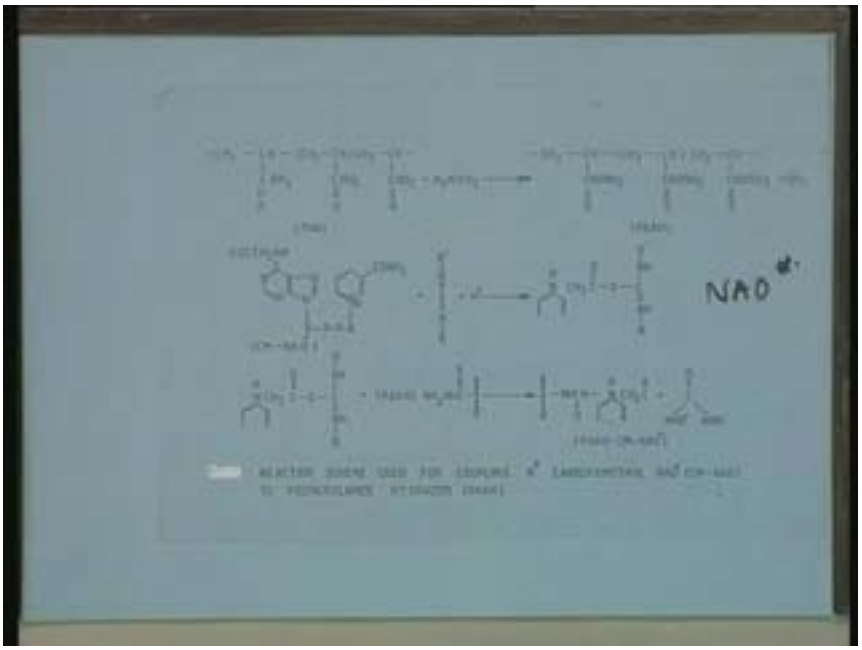

This activated NAD is coupled with the polyacrlyamide hydrazide and this on coupling gives you a water soluble large molecular weight coenzyme analogue which is functionally compatible with the dehydrogenase which you want to use and similar systems can be designed for use.

This describes a typical laboratory setup in which a stirred cell type of ultra filtration system has been used. A is the feed reservoir; it is passed through a valve which can direct the reaction vessel either to the reservoir or to the cylinder for pressure. It can have both the directions and the reaction can be carried out high pressure and the reactor contains the polymer bound co-enzyme, the substrate and the two enzymes. We are carrying out reaction in CSTR and when the reaction goes on, the product formed is sent out as the permeate in the ultra filtration cell and the enzyme and the polymer bound coenzyme is retained in the reactor and the substrate can be fed continuously by pressure gradient in the reactor vessel and the reactor can be continued over a long period of time.

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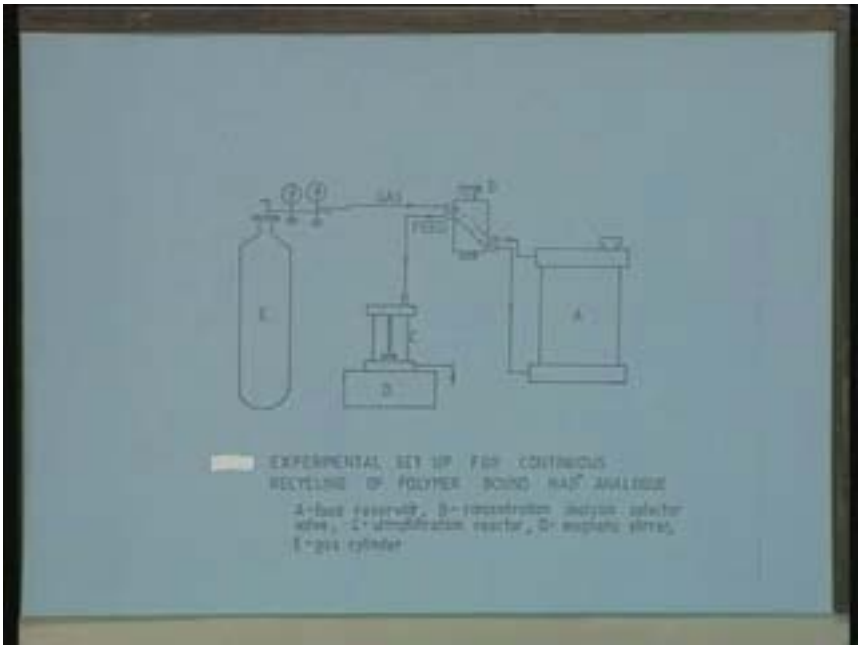

Two of the reactions which have been carried in such a reactor is one is continuous oxidation of ethanol using permeabilized yeast cells. The yeast cells when they are permeabilized, the lipid on the membrane is dissolved by a solvent and it becomes freely permeable to various substrates. That means it has lost its permeability barriers for substrate and product exchange. In fact it has shown to give some kind of enzymatic activity which can be called as NADH oxidase; it oxidizes the reduced NAD into oxygen like an oxidase activity and it generates hydrogen peroxide and this hydrogen peroxide to break down requires peroxidase or else it will inactivate the original enzyme alcohol dehydrogenase.

So in the same reactor system if you use ethanol, polyacrylamide hydrazide coupled NAD and peroxidase you can get a net conversion oxidation of ethanol to acetaldehyde and the profiles shown here give you the kind of reaction progress. This is reaction progress in hours. In the first profile there is no peroxidase added which means as soon as the reaction starts the hydrogen peroxide produced inactivates the alcohol dehydrogenase and no further reaction takes place. Only when you add hydrogen peroxide, here hydrogen peroxide or peroxidase has been added, you get the reaction going on to a sizable extent until the time again because of some reasons either the enzyme has leaked out and the alcohol concentration again builds up and there is no reduction.

In third case the system was improved by treatment of the alcohol dehydrogenase containing cells by glutaraldehyde which is aimed to fix the alcohol dehydrogenase on to some cellular organelles and there by making it almost in the form of an immobilized enzyme so that it doesn't leak out and you can see the clear advantage that the reaction is extended over a large period of time comparatively but again still it goes and although this system can be demonstrated for eight hours or ten hours but still there are some problems which are still so far not understood as to what happens in the case of cofactors cycling.

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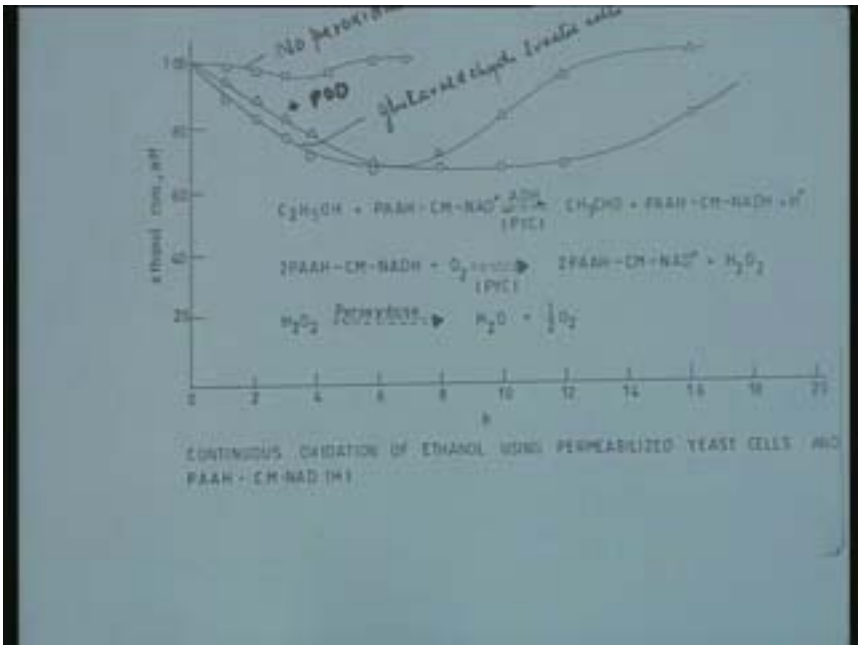

The concentration of NAD here is very, very small and therefore the cycling is taking place; there is no doubt. But because you are ultimately here reducing the concentration let us say from hundred milli molar to an extent of about seventy milli molar which means thirty milli molar concentration has been reduced which means that in a NAD concentration of let us say ten milli molar, seven hundred cycles have taken place. But ultimately this cycling is not continuous over a long period of time and that is still inherent problem.

A similar profile is observed in the case of conversion of pyruvate to alanine using same polyacrylamide hydrazide coupled NAD and formate dehydrogenase and alanine dehydrogenase. As I mentioned the use of format dehydrogenase has an advantages that the reaction products are carbon dioxide and water which can be easily stripped off but you have to provide formate as a substrate along with it and the circles indicate no glutaraldehyde treatment and the triangles is with glutaraldehyde which very clearly indicates that the down fall is prevented and the enzyme is available over a longer period of time and reaction continues. But the data beyond sixteen hours have not been shown here but within a couple of hours definitely not more than twenty four hours it is able to say and the reactor performance drops down.

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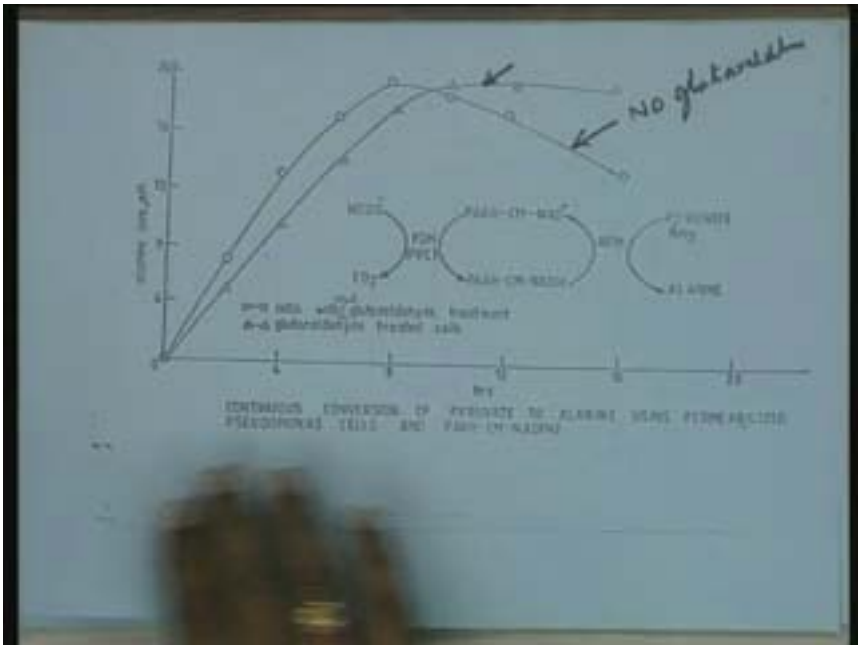

The second issue was to recall bio processing of unnatural substrate. Here we had highlighted that there are a number of enzyme reactions which although give you lot of benefits, very favorable concepts, in terms of their specificity, in terms of the desired nature of the product they produce in the right stereoisomeric form but still the problem is also in relation to the never specificity gives you a much more problem.

If you look at the two reactions that I referred to earlier that is conversion of fumaric acid to malic acids and also maleic acid to aspartic acid. Both reactions involve addition of water and ammonia respectively on to a carbon, carbon double bond and give you the corresponding products malic acid and aspartic acid. Both the products find application in the food or related industry. Malic acid is directly used in most of the beverages as the acidulant and aspartic acid, the bulk use is in synthesis of aspartane but the quantity required is not very large.

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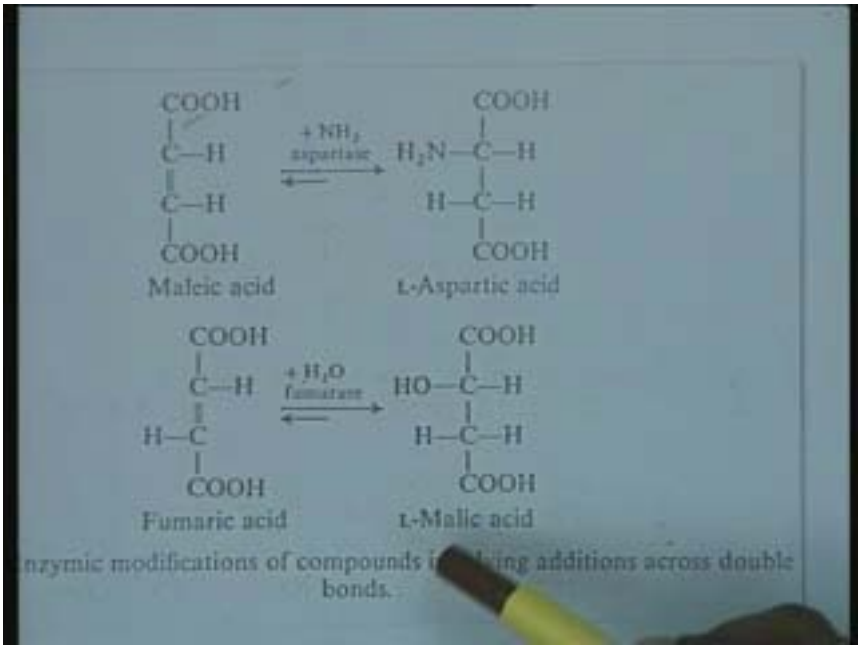

On the other hand look at similar reactions which are of commercial value and in large quantity. They are just representative reactions; there are many more reactions which come under this category.

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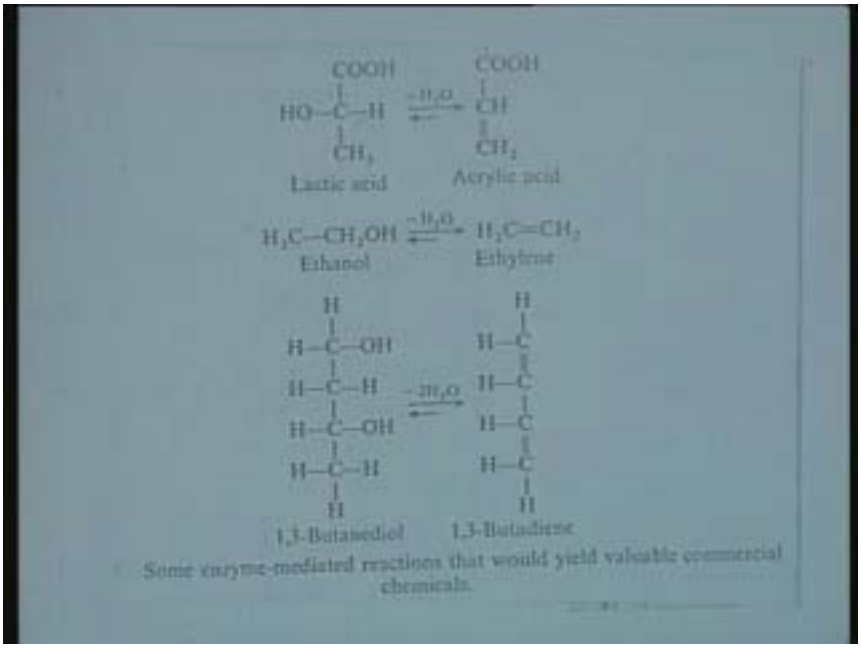

You see lactic acid to acrylic acid. Removal of water molecule from lactic acid is reverse of what fumarase does and this reaction is of immense value and if it can be carried out it can serve a great purpose. Similarly extraction of water from ethanol to give ethylene or dehydration of ethanol. Then again extraction of water from 1,3-butanediol to produce

1,3-butadiene which is a substrate or monomer for synthetic rubber. All the three substrates which are listed here are bulk fermentation products. If they can transform enzymatically using an enzyme something similar to that of fumerase or a modified fumerase probably one can have very significant impact on the enzyme technology. Such efforts are drawing attention of people so that we can design enzymes reactions to meet the requirements. In general although the enzymes catalyse very desirable chemical transformations like fumaric acid to malic acid or maleic acid to aspartic acid but often they convert only substrate that have limited commercial interest.

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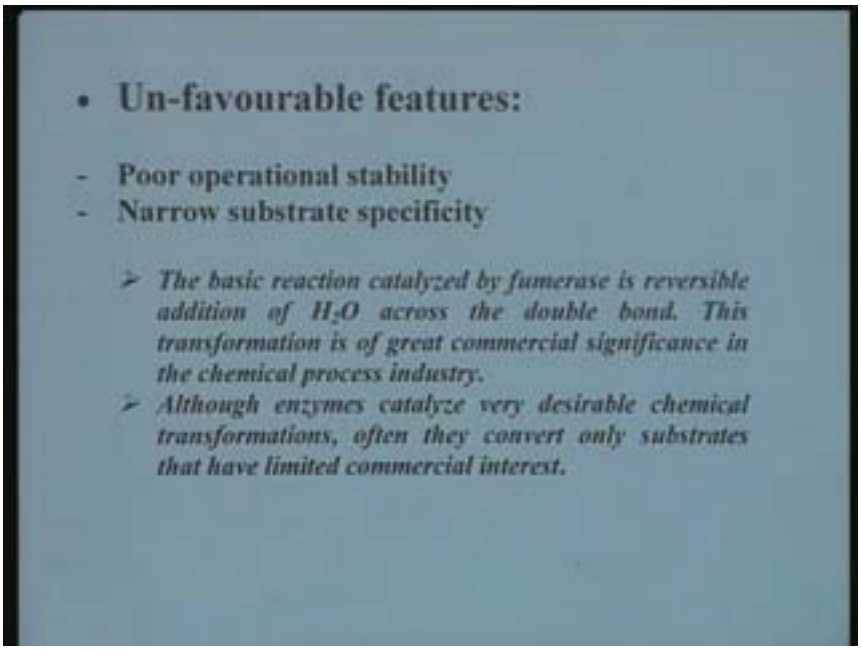

In fact if these enzymes can be used for those conversions which have very varied and very large commercial interest the impact will be much greater and one can be really fascinated with the industrial application of enzymes.

Other feature was people tend to accept the enzyme names very literally and in absolute sense. When we name glucose oxidase we interpret that glucose is a substrate with oxygen as the ultimate lactone acceptor for oxidation of glucose. Similarly galactose oxidase also is in the same concept but in practice these enzymes are not so highly specific.

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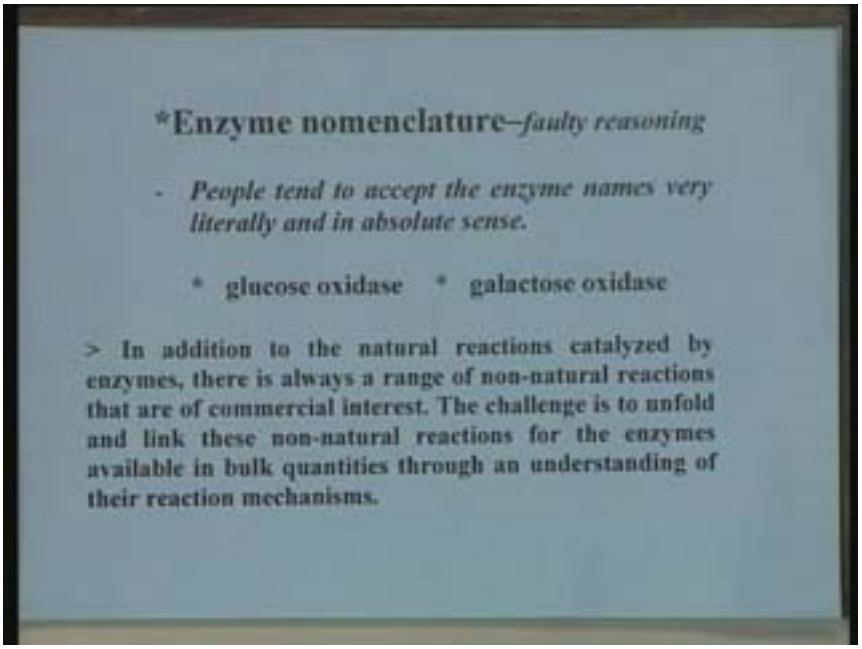

In the first slide we saw that some of the enzymes which are of industrial value are very specific. They have very narrow specificity and we need to broaden their specificity. On the other hand we have certain enzymes which are broadly specific which are not very narrowly specific but we are not able to use them for variety of reactions. So both the things have to be really tackled so as to be able to apply enzymes on a large perspective like for example glucose oxidase can use large number of electron acceptor other than oxygen which can be then reduced at the expense of glucose. Glucose will get oxidized and the corresponding electron acceptor will get reduced and one can carry out a number of organic chemical transformations.

Galactose oxidase is another enzyme which is known to catalyse the classical reaction of galactose to the corresponding aldehyde by oxygen. But this galactose oxidase is a very broad …… applicable enzyme and it really acts on a variety of aliphatic and aromatic alcohols to give rise to corresponding aldehydes and these aldehydes, the conversion of alcohol to aldehydes oxidation reactions, are of great significance in perfumery and cosmetic industry and a number of reactions can be catalyzed. One thing you must be sure is that the rates on different substrates might be different. Today with an understanding of the site directed mutagenesis, it is possible to change the substrate specificity and also improve the catalytic functional rates.

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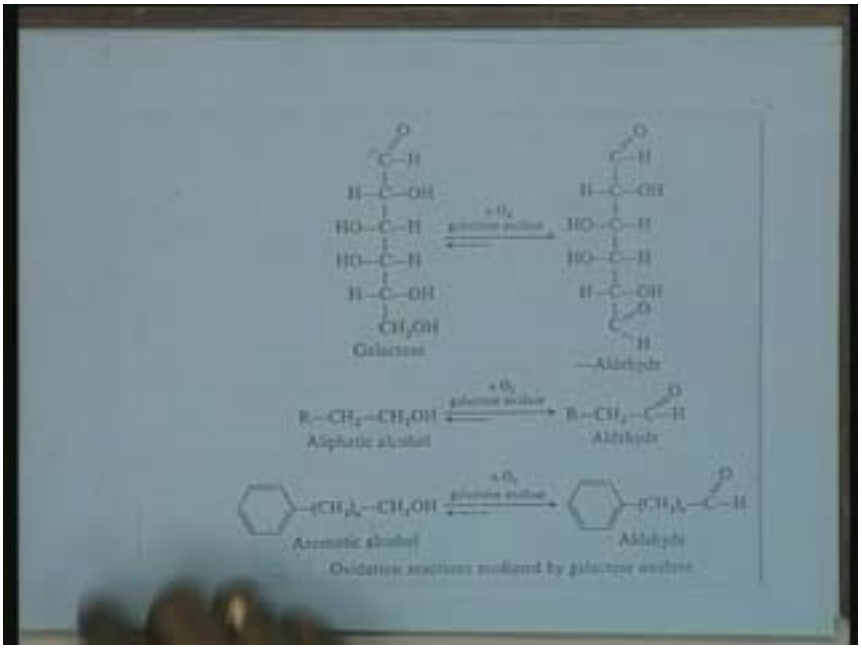

So the message is that in addition to the natural reactions catalyzed by enzymes there is always a range of non-natural reactions that are of commercial interest. The challenge is to unfold and link these non-natural reactions for the enzyme available in bulk quantities. Another feature is first we must pay attention to those enzymes which are already available in bulk quantities.

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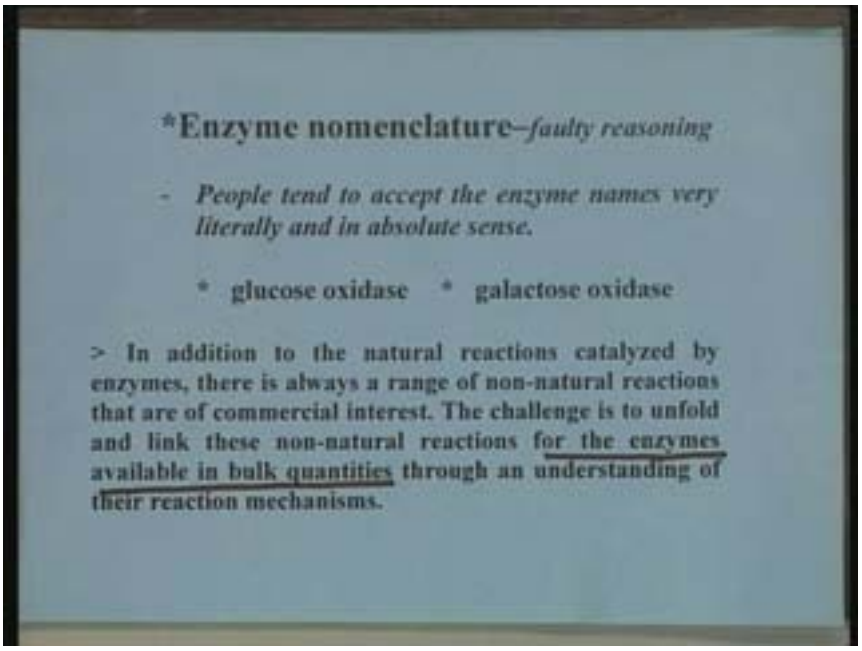

Because one would like to look at a particular bio transformation but if the enzyme is not available in bulk quantity at a low cost the application may be inhibitory; may not be

feasible. So best is to initially look at those enzymes which are available in large quantities and one can only diversify their application to a large range of non-natural reactions which are of commercial value and this will require very clear understanding of their reaction mechanism because presently those reactions are being carried out by chemical transformations and making use of the reaction mechanism one can probably think of certain unconventional reactions.

The third interesting area which I wanted to talk was bio-organic synthesis using enzymes in micro-aqueous medium.

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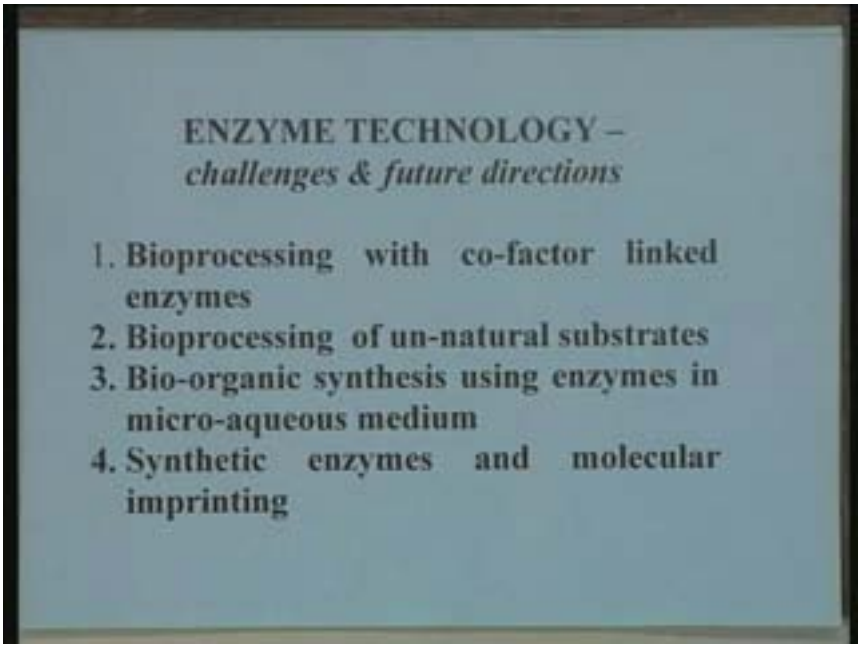

Normally we understand that enzymes are operable in aqueous medium and the basic reason is that a large number of non-covalent interactions that we are familiar with enzyme structure are through interaction of the enzyme protein with the water molecule and water is a requisite specie in the case of maintenance of a confirmation of an enzyme molecule and to carry out the chemical or bio chemical reactions. Such a perception in the earlier days was based on a very limited understanding of the enzyme structure and function and the limited understanding was that people had looked at the stability of the enzyme in water miscible solvents say for example acetone which is still used for precipitation of enzyme proteins; it makes aggregates and precipitates; dioxanes which are water miscible solvents and beyond certain concentration of these solvents in the bulk medium inactivates the enzyme.

Now people understood that enzymes cannot be used in the non-polar environment which ultimately proved wrong in atleast for the last twenty years and people have looked at a number of water immiscible solvents which are known to keep enzyme in a very stable confirmation along with the maintenance of enzyme confirmation because the quantity of

water required for interaction with the enzyme protein is a very small bound water molecule around the external surface of the molecule which is retained and which is not very easy to be removed by normal dehydration methods. Unless you subject it to chemical treatments you cannot really remove it by conventional drying methods the bound water present in the enzyme molecule. In the recent years number of applications have already emerged but on a limited scale and probably the scope it offers is much, much larger than it is being practiced today.

Some of the advantages of organic media for bio-catalysis are: one is many of the reactions which require non-polar substrates lipids mainly are very difficult to carry out in aqueous medium because they are not soluble or the concentration that will be soluble will be very small. So if you want to carry out those reactions in high concentration you need a non-polar bulk medium and the substrate can be dissolved and then they can be used for enzymatic catalysis.

Another major advantage is the hydrolysis can catalyse synthetic reactions. That means in the absence of water for hydrolysis which require water as a second substrate or for hydrolysis reaction water is the reactant. If the water is stripped off or is not present then the reaction can go in the reverse direction and instead of hydrolysis, synthesis can be used and in fact it is in this area a very significant application have emerged where the ester synthesis has been carried out even commercially in fact for some of the very high value products.

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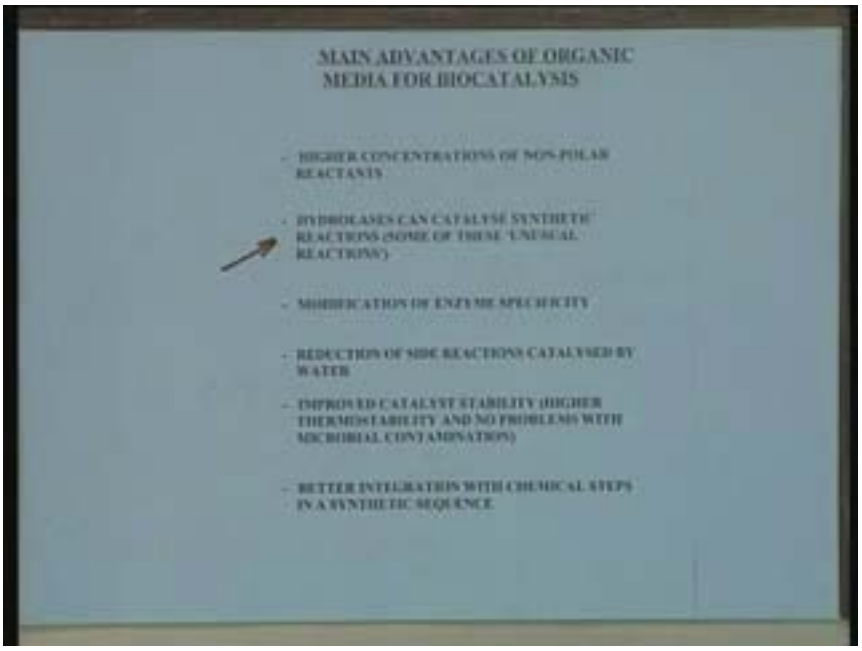

Particularly, more importantly for resolution of racemic mixtures which we know that the enzymes are able to recognize one of the isomers the resolution can be carried out in the case of chemically synthesized racemic mixture.

Then many of these can be used for modification of enzyme specificity as far as the reaction is concerned in the organic medium. It has been also seen that when you subject enzyme molecule into anhydrous organic solvent the enzyme becomes very rigid. If you put an enzyme molecule into aqueous media, in buffer the molecule in highly mobile. It interacts with the water molecule freely and it is very mobile but when you put in organic non-polar medium its mobility is drastically reduced. In fact one of the major Scientist working in this area he has called these as rocks in the organic solvent. The molecule becomes so rigid that the structure is very rigid and deactivation is impossible. The molecule becomes rigid; it doesn't deactivate it retains its activity and that is one of the advantage and during this rigidification lot of modification in the substrate specificity also occurs and this has been noted in many cases and coincidentally bulk of the studies in use of enzymes in the non-polar medium has been in the area of hydrolases mainly lipase and proteases. They are the two classes of enzymes lipases and proteases which have been extensively studied for variety of reactions. I will illustrate with some of the examples.

Then reduction of side reactions catalyzed by water. When water is not present any reaction catalyzed by water is inhibited. Then higher stability both thermal stability and no problems with microbial contamination because the solvent itself will act as a preservative and not allow any microbial contamination to take place and the enzyme can be stored over a long period of time. Even operational stability is improved; as I mentioned that the confirmation becomes very rigid. It's not possible to alter it during reaction and the mobility is lost. Another major important factor is better integration with the chemical steps in synthetic sequence where you want to use enzyme as a part of a sequence of reactions in an organic chemical synthesis where the substrate is coming already dissolved in organic solvent you can always integrate enzymatic reaction in the sequence of steps. Otherwise it was difficult earlier because if the substrate is coming in the solution form in an organic solvent you need to remove the organic solvent put into water medium and then carry out the reaction which is practically not feasible but which can be made feasible if you use the advanced enzymes in the organic medium.

Some of the alternative ways in which enzymes can be used in the organic solvent are shown here. If you look at the first one, the 'A', it is a homogeneous system containing individual enzyme molecules, water and water miscible organic solvent. Typical example is acetone, water mixture. You take ethanol water mixture put the enzyme carry out the reactions. Such a system practically is not very, very attractive because as I mentioned they tend to inactivate the enzyme because water miscible solvents often tend to extract the bound water layer of the enzyme and thereby the confirmation is disturbed. The second is the use of enzyme in two phase systems; an organic and water two phase systems where the enzyme is present in the aqueous phase, the substrate is present in the non-aqueous phase; make an emulsion by agitation or use of surfactant, bring the enzyme and the substrate in close proximity and then carry out the reaction.

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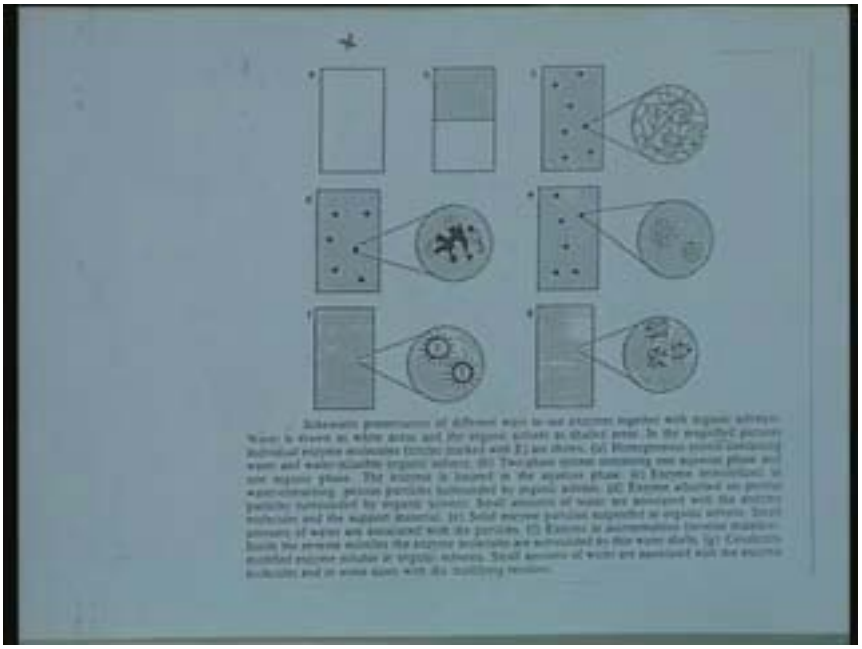

In the third configuration. ‘C’, the enzyme is immobilized on water containing porous particles surrounded by organic solvents. That means you use a hydrophilic support and the hydrophilic support will contain the enzyme particle immobilized along with some water molecules dispersed around it and the whole particle is then immersed as shown in a magnified way here. The enzyme particles which are hydrophilic contain the water molecules around the enzyme and they are suspended in the bulk organic medium. The shaded portion indicates the organic medium and the blank spaces are indicated by water medium and the enzyme particles are marked E.

The fourth configuration is the immobilization of the enzyme particle on a hydrophobic matrix. There is no water present in the proximity of the enzyme molecule and the hydrophobic matrix and the enzyme is immobilized in a hydrophobic patch and it is suspended then in the organic solvent. The fifth one is solid enzyme particles suspended directly. You take enzyme particle, dry it and suspend into solvent and because they are not soluble they will tend to form lumps, aggregates that is what these aggregates are shown and they can be used to carry out the reaction. Then the sixth one is you make reverse micelle by use of surfactant. You take enzyme in the micro emulsion form and inside the reverse micelle you have the enzyme entrapped in the water cells. That means the enzyme experiences an atmosphere which is hydrophilic in nature but the whole reverse micelle is surrounded by the organic solvent. This is the enzyme entrapped in the reverse micelle. Reverse micelle is just like water in oil emulsion where by you have enzyme entrapped. Within the reverse micelle the amphipathic molecules make a structure where by all the hydrophobic tails extrude outwards towards the bulk organic solvent and the polar heads are all made into a small network within which the enzyme particle is trapped and some water is also entrapped within the cell and therefore the enzyme remains stable almost as if it is experiencing an aqueous phase. But the whole particle, as a micelle particle is immersed in the organic phase.

Then you have the last configuration in which enzyme is modified by chemical modification so as to be made soluble in the non-aqueous phase. One of the well known reported methods is modification of enzyme by coupling to polythene glycol. If you couple polythene glycol, the whole enzyme molecule becomes soluble in non-aqueous phase and then it can be put into an organic medium and then it behaves almost like a soluble enzyme but in non-aqueous phase. But again doing covalent modification of this enzyme, you loose lot of activity and there might be some change in the conformation so as to alter substrate specificity in the process.

If you look at the structural understanding of the enzyme what happens when we immobilize either on hydrophilic or hydrophobic matrix? Most of the enzymes in organic phase are used in immobilized form. If you look here this is a free soluble enzyme. As I mentioned earlier the enzyme molecule is surrounded by water layer and this water layer is essential for the enzyme conformation and activity. When the bulk solvent is water and the continuous phase is water there is no disturbance on this surrounding layer and the enzyme remains stable for a reasonable period of time. Due to thermo chemical reasons it might get inactivated but for some time it remains intact.

When you immobilize the enzyme on to a solid matrix, let us say a polymeric matrix, it could be hydrophilic or hydrophobic matrix you generate an interphase with the bulk medium and the polymeric matrix is surrounding the water layer which is maintained during the reaction. No. Bulk here is organic solvent and here it was water. In the third case it is a reverse micelle. Here also you have an interphace which consist of hydrophobic tails.

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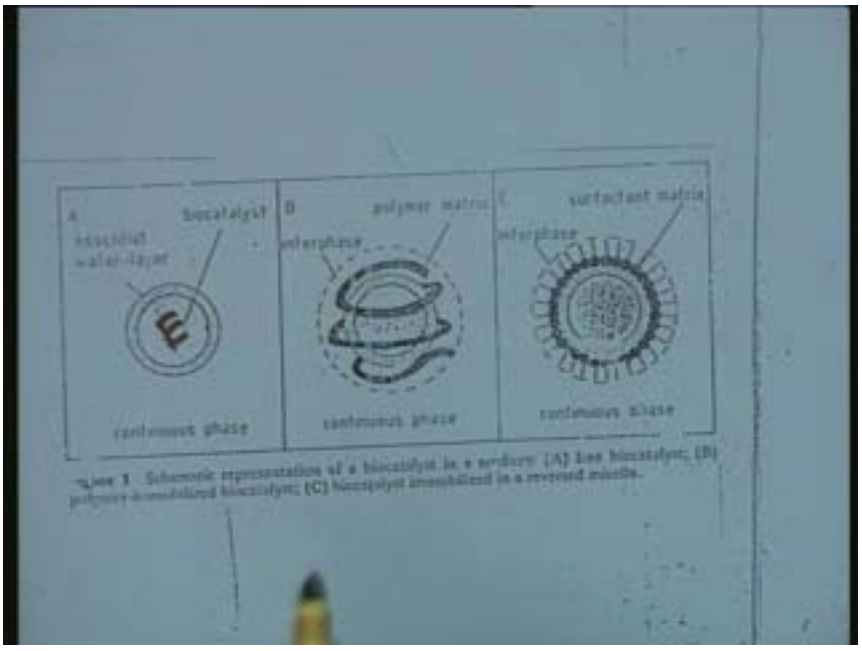

The fatty acid or whatever lipid molecule you have used for making the micelle, they are arranged such that the hydrophobic heads are diverted inside which form a structure and tails are extruding outside and then you disperse it through the use of surfactant and you have an interface here consisting mainly of the hydrophobic tails which remains very stable in the organic medium. Although this provides a very neat system for carrying out enzymatic reaction in organic phase, the only problem is the recovery of the product, after the reaction is over, from the system. Recovery of product means you need to disrupt the micelle, reform the micelle so the reaction cannot be really carried out in a continuous mode and that causes severe problem, operational problem. But otherwise as a system if you look at this provides you an ideal system because it gives you very large interphase; it gives you a hydrophobic interface which can remain stable in the ……. and also enzyme is very stable because it is experiencing the aqueous environment.

The important parameters for the enzyme reactions in organic solvents are the source and presentation of the enzyme. As I mentioned the variety of forms in which the enzyme can exist either as a free enzyme, immobilized enzyme, soluble conjugate, that is …… enzymes or in microemulsion form either of the way; that is one parameter and one need to look at the kind of form of the enzyme that gives the best results. Then the choice of solvent is another feature. It has been noted in most cases that the solvents which are relatively more non-polar are better suited for carrying out enzymatic reaction mainly because of their lack of interaction with the water layer on the enzyme molecule. In fact people have defined as far as the choice of solvent is concerned a parameter what they call as log P where P is the partitioning coefficient of the solvent between a particular solvent and octenol.

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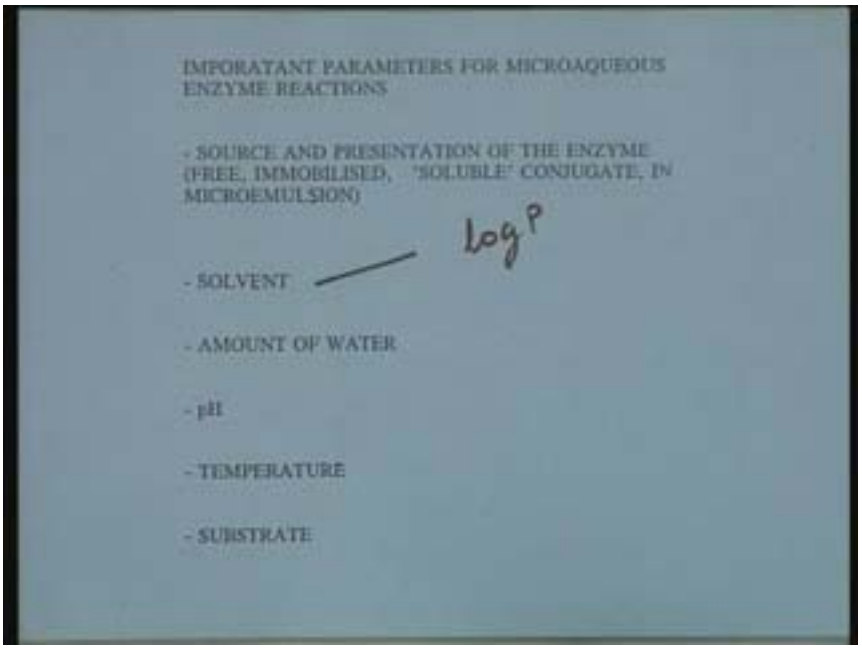

Octenol is considered to be the extreme non-polar medium and the partitioning coefficient of that solvent between water and octenol is the P value and higher the log P value means it is more non-polar and higher the log P value the better it is a suited solvent for enzyme catalyst. They do not interact with the essential water layer on the enzyme molecule because of their non-polar nature and therefore the enzyme structure is maintained.

A very important factor is amount of water present.

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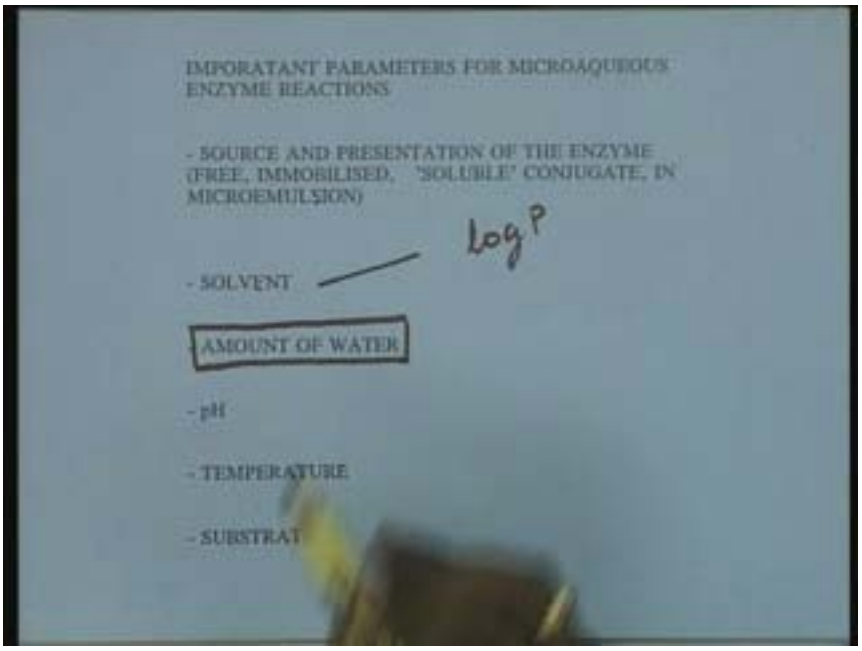

Almost all the reactions when we want to carry out in organic solvent we also need a very small quantity of water which plays a key role in the catalytic function of the enzyme. It is a question of debate whether this amount of water required is to maintain the essential water layer in equilibrium or there are some other catalytic functions which require water molecules by the enzyme because so far if you look into reaction mechanisms of enzymes excepting for maintaining the enzymes structure, water has not been seen to play any role. But once if we say that you have a very high log P value of the solvent which has no miscibility with the water it will not disturb the essential water layer but still a small quantity of water is required in the organic solvents to maintain the catalytic function and there are number of approaches which has been made to provide that small quantity of water and also to strip the water produced in the reaction. When you talk of hydrolysis being used in the reverse direction for synthetic purposes water will be produced and this water needs to be essentially removed. On the other hand there is an optimum water concentration which is required but when water is produced water has to be removed rather being added.

Then pH is another feature. When you carry out the enzyme reaction in an organic solvent the role of pH becomes limited in the sense that as we saw earlier in the effect of pH in enzyme kinetics, the major role was ionization of the amino acid residues in the enzyme protein. A non-polar solvent will not really allow ionization to take place and therefore pH theoretically must not play any role. But the role of pH in the case of enzyme reaction in organic solvents has a different connotation and the connotation is that it has been reported by some workers that enzymes unlike many other small molecular weight chemical compounds possess a memory with respect to certain physical features say for example pH. For dispersing the enzyme into an organic solvent you are starting from enzyme which is already dispersed in some aqueous medium and whatever pH was of that medium, the enzyme retains that pH in the memory and the functional aspect of the enzyme when it is transferred to non-polar medium remains at the same pH. So you can make different enzyme preparations starting from initial pH in the water medium which have different functional capabilities and by changing the enzyme memory with respect to pH. Similarly temperature and nature of substrate also are important parameters and they have the similar features as we saw in the case of water in aqueous medium.

Just to illustrate one of the reaction system which we did some work here in IIT itself and the enzyme reactors systems were developed here and used for a number of reactions which I have skipped. It consists of stirred reactor in which silicon tubing is dispersed into the organic phase and silicon tubing, while it is non-porous to most of the substrates and the products, but it is porous to water vapors and what we do is through silicon tubings we recirculate a saturated salt solution which is known to have a definite water activity. In fact for calibration of the humidifiers we use saturated salt solution and each salt, when you make a saturated salt solution has definite relative humidity and those salts are circulated so as to maintain a constant water activity in the reactor and even control water activity.

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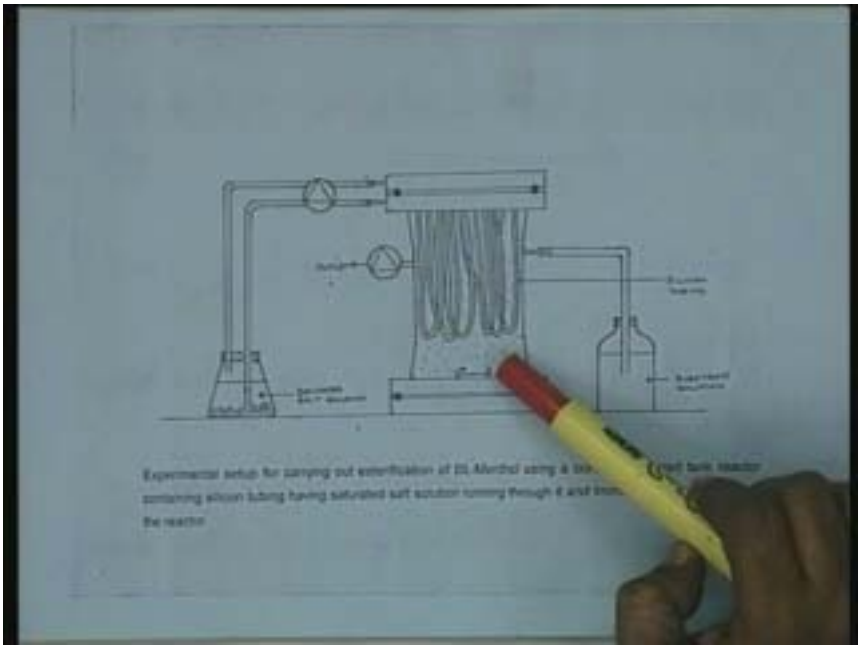

That means this saturated salt solution if more water is produced will take up water and when water will get depleted it will act almost like a water buffer just like a buffer for acids and bases, this acts like a buffer and one can control water activity in organic reaction system and carry out the reaction at a constant water activity.

A number of enzyme catalyzed reactions have been reported like lipases for ester synthesis, interesterifications, transesterifications and resolution of racemic mixtures. Because each enzyme has specificity towards one of the isomers, substrates can be resolved into respective isomers. Proteases for peptide synthesis, oxidases for variety of reactions for example cholesterol to cholestenone, butanol to butanaldehyde, p-phenylphenol to phenol-formaldehyde resins, p-cresol to o-quinone and ethanol to hydrogen peroxide. Even transferase have been used in organic medium for sucrose to levan, a polymer and glucose to fructose isomerisation has been shown to shift the equilibrium constant from fifty percent equimolar in the favor of fructose while when we use in the non-aqueous phase.

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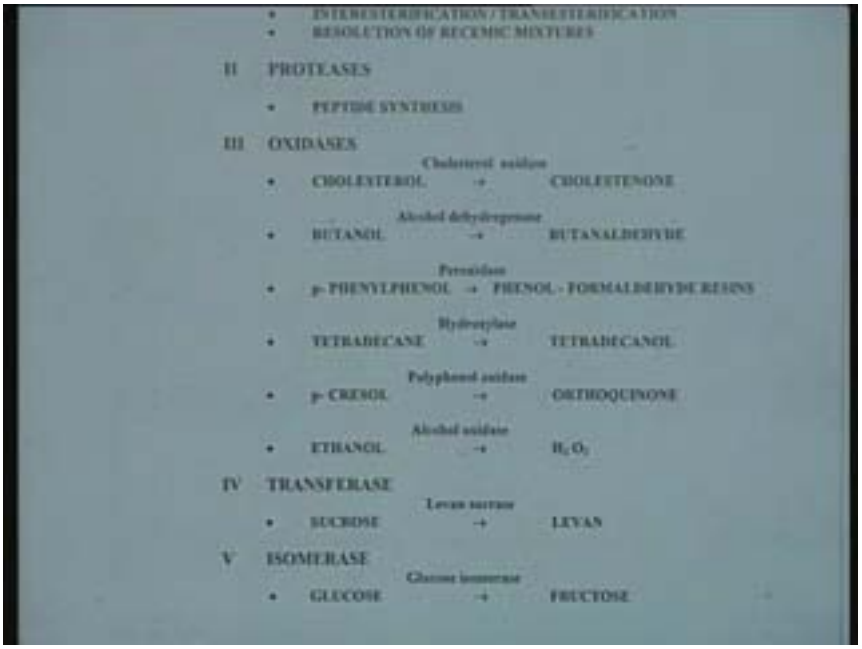

Last feature is synthetic enzymes in molecular imprinting. We know that the enzymatic catalytic function has two key roles: one is binding of the substrate and then certain catalytic function.

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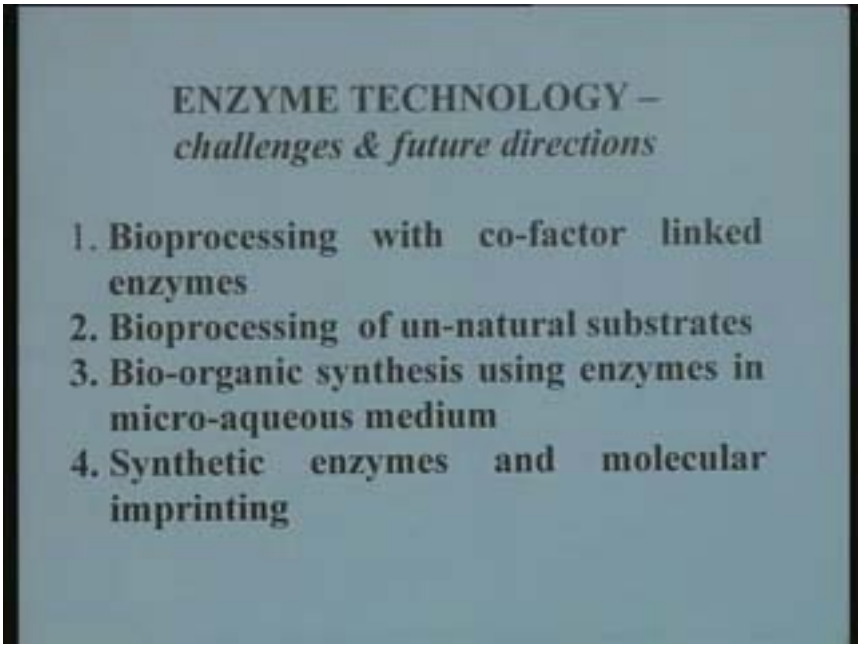

So what we need in the case of synthetic enzyme is that we must have kind of a large molecule with a cavity so that a substrate of definite size can fit into it and mimic a binding process. The other is in the proximity of the cavity there must be some functional groups which can do catalyses and we know that the enzymes also follow the same kind

of reaction mechanisms as chemical catalysts do or acid base catalysis, covalent catalysis, or proximity effects; all those there are experienced in the enzymatic reactions and the similar concepts are being used to synthesize large molecules to mimic the catalytic functions. In fact one of the most successful attempts has been to use cyclodextrins. The cyclodextrins are cyclic molecules with six, seven and eight glucose moieties coupled together in a starch.

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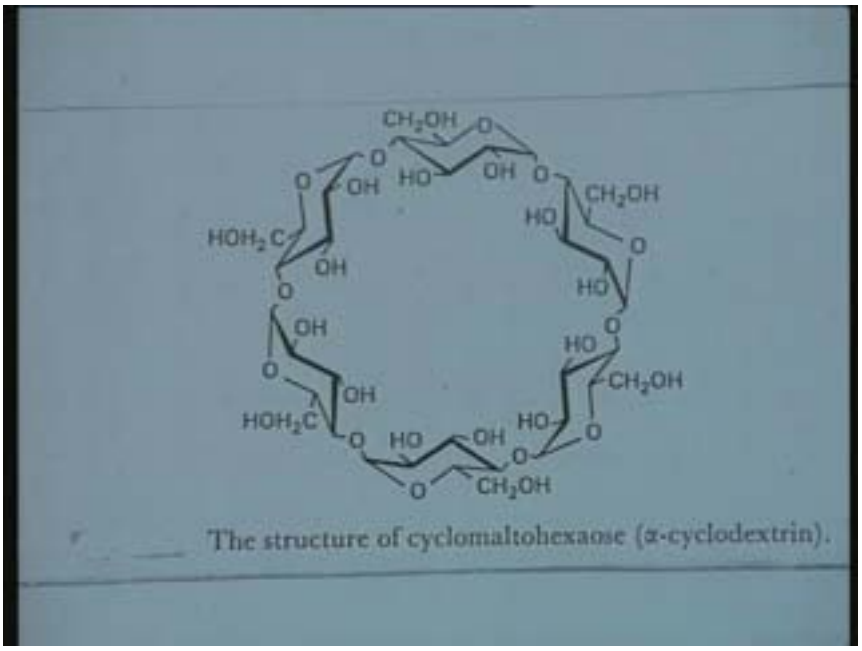

That means α-1,4-glucoside linkage. If you degrade starch you will get dextrins and these dextrins if they are cyclic molecules we call them cyclodextrin and the alpha, beta, gamma cyclodextrins are six, seven and eight glucose molecules linked in the cyclic form. The peculiar feature of this is that the cyclic molecules make a cavity of definite size in which you can retain the definite molecules and this cyclodextrins are being used to attach by covalent coupling some of the chemical groups which can catalyse the reaction.

If you look at the cavity of cyclodextrin it is almost like a paper and cylinder and on the surface they attach a number of functional groups, the same functional groups which are known to be possessed by and this is for example chymotrypsin analogue. They have tried to mimic the activity of chymotrypsin; they have named it also benzyme and some of the functional groups are covalently coupled which are functional in the case of catalysis and such a complex is called a synthetic enzyme.

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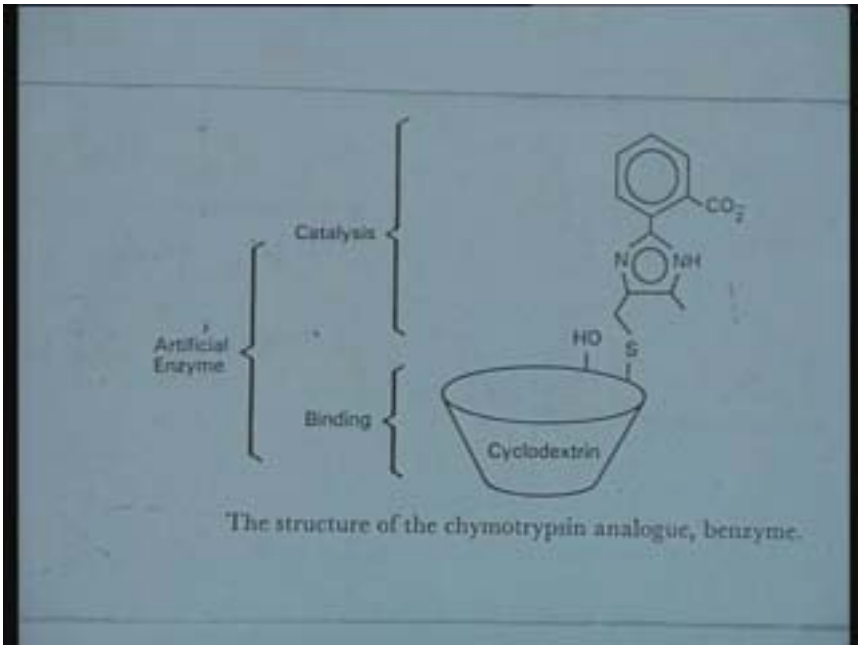

The structure of the chymotrypsin analogue, benzyme.

Many such molecules are likely to come out. But so far not many synthetic enzymes are available which can carry out diverse function as the normal natural catalyst do.