ENZYME SCIENCE AND ENGINEERING

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LECTURE – 1

INTRODUCTION AND SCOPE

This course as you know, deals with one of the largest group of biomolecules what we know as enzymes. You are familiar with number of biomolecules like carbohydrates, lipids, proteins. This enzyme falls into the category of proteins and the course will cover various aspects of enzymes particularly in relation to their application as a biocatalyst in the process industries.

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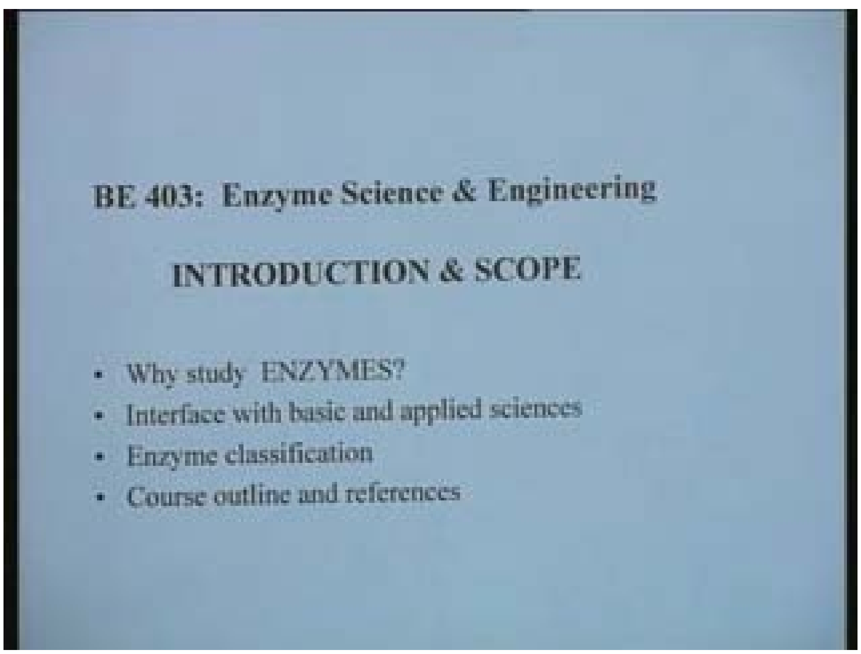

I think one of the very basic issues which come to mind is that why do we study enzymes as a separate class? In addition to your study of various biomolecules in other courses like Biochemistry, we have separate course on enzymes and some of these aspects I will be dealing in today’s lecture which will basically be of introductive nature and also give you the scope of this course.

The first issue as to why we study enzymes opens up many features of enzyme molecules and the first and probably the most important is their role in a living cell or their role in the realm of biotechnology as a whole. If you look at the basic definition or a very broad operational definition of biotechnology it deals with the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services.

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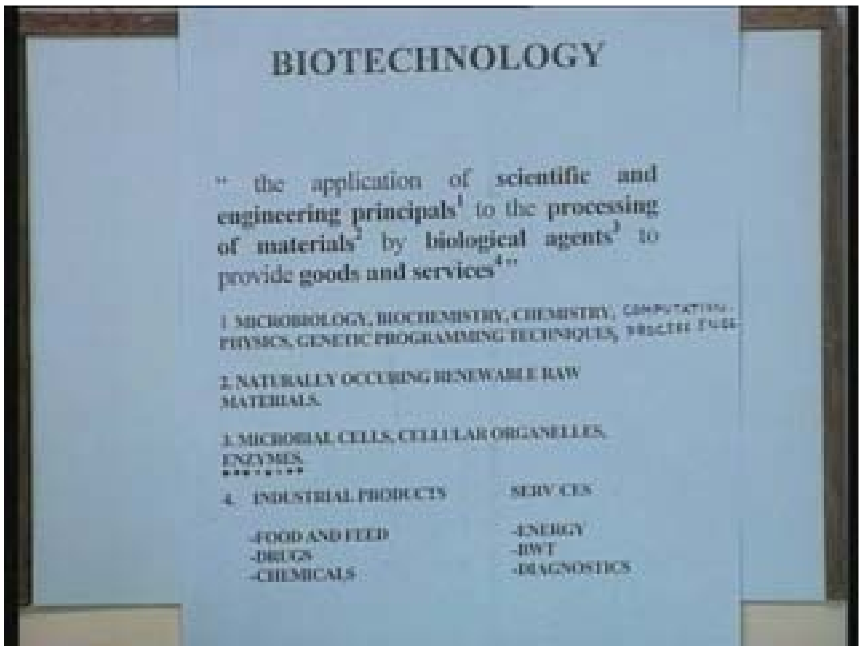

Here I like you to mark the term biological agents and these biological agents cover microbial cells, cellular organelles, enzymes, may be other parts of the cells like membranes in a variety of biological material that can be used as a catalyst in process industries. That is the upcoming feature of biotechnology and among these groups of biocatalyst enzymes play a very significant role. If you go a little deeper into the various activities of a living cell besides looking at the industrial application, a living cell is a very complex entity. It carries out all functions of any chemical process plant that you have known or you can conceive. Most complex chemical processing plant say for example petrochemical complex where the petroleum is fractionated into various products and I like to give you a very simplified analogy of a living cell to that of a chemical process plant. The cell does a variety of functions that is crushing and grinding, transport of materials, power generation, control of information, transmission of information and then release of finish products to the user client.

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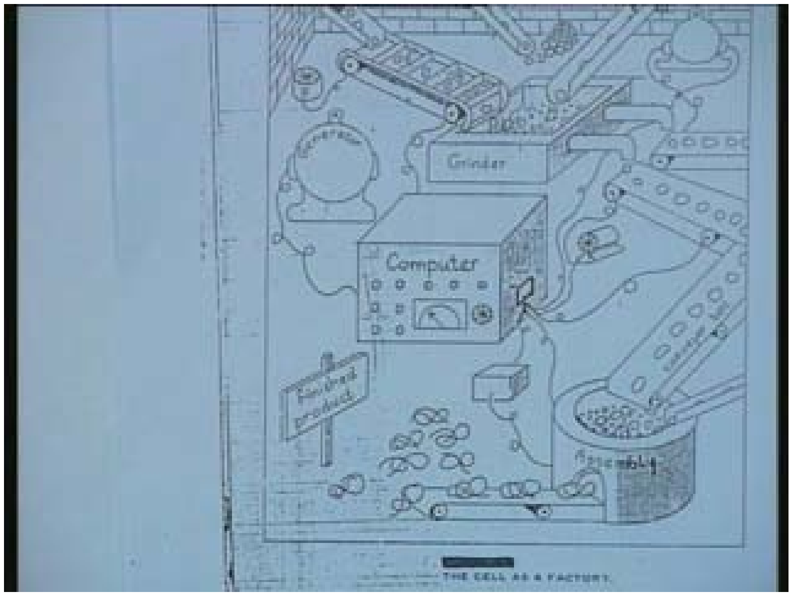

All these functions are also basically carried out by a living cell. Now the key issue is that all these functions in the living cell, may it be a microbial cell, may it be a plant cell or it might be an animal cell, they are mediated by the catalyst known as enzymes. Therefore the enzymes play a very central role in the function of living cells or in other words you can say that they are one of the most important executives in a living cell. Even disturbance in one of the enzyme catalysed reactions can lead to some abnormality in the cell function.

We are familiar with many of the diseases which are seen when one of the enzymes stops functioning. Similarly in case of a living cell if the enzyme system fails many of the processes can be disturbed ultimately leading to the life cycle of the cell itself. It is such an important component it becomes important to understand how they perform those reactions and how they manage the integrity of whole cell. The most significant part is that they regulate the various functions of the cell in such an accurate manner probably no other control system in any chemical process plant can think of.

There is a perfect control, fine tuning of the enzyme activities as per the desired requirements and we also know that the cells are energetical, in terms of material consumption are very efficient systems and wastage is minimum. The energy is generated to the required level; materials are stored in case they are not needed. All those functions are a key part of the living cell and all these functions are attributed to the function of enzymes and that is where we get to understand enzymes in a more detail fashion. Also we must appreciate that the cells, making these functions possible, carry out a large variety of reactions. They can range from hydrolysis to polymerization. We are all familiar of how the different components or molecules or intermediates in a living cell are synthesized. They all take place through a series of chemical reactions.

These reactions could be hydrolysis could be polymerization, could be epoxidation, could be reduction and so on. A whole lot of chemical reactions do take place in a living cell.

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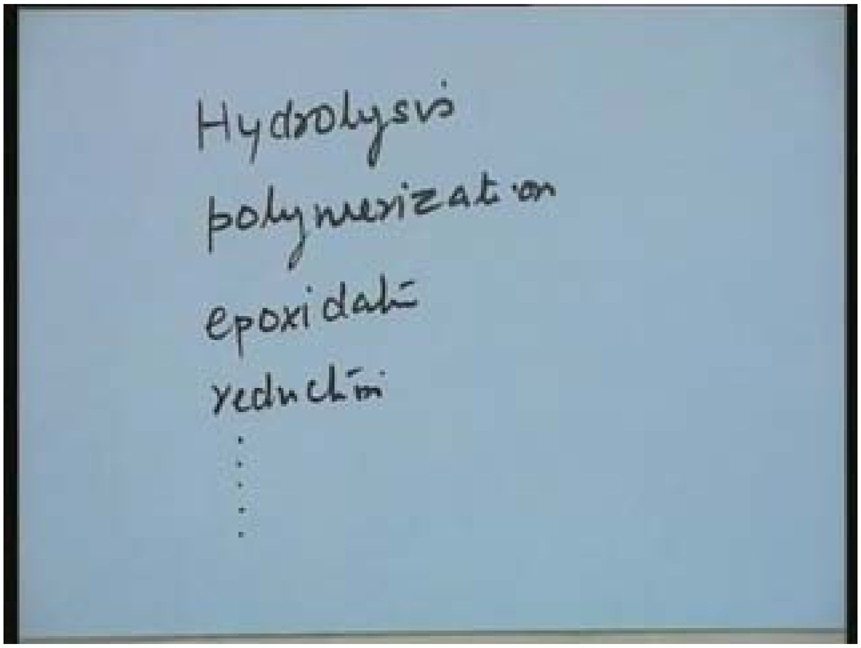

The same scenario is that even a chemical process industry depends on a whole lot of chemical reactions which are catalyzed either spontaneously or with the use of catalyst. The analogy lies that the reactions that are catalyzed by the enzymes in a living cell if they are carried out in an industrial atmosphere with perfect energetically economical situation, environmentally friendly conditions we have a good friend in enzymes and that is again another basis of use of enzymes in the process industries and also the possibility of studying them in such a great detail.

If you look at some of the aspect of enzymes and the process via catalyst, a very broad picture which refers to the role of enzymes as the bio catalyst, we must be aware that all these enzymes functions at the ambient conditions at the conditions at which living cells are known to live.

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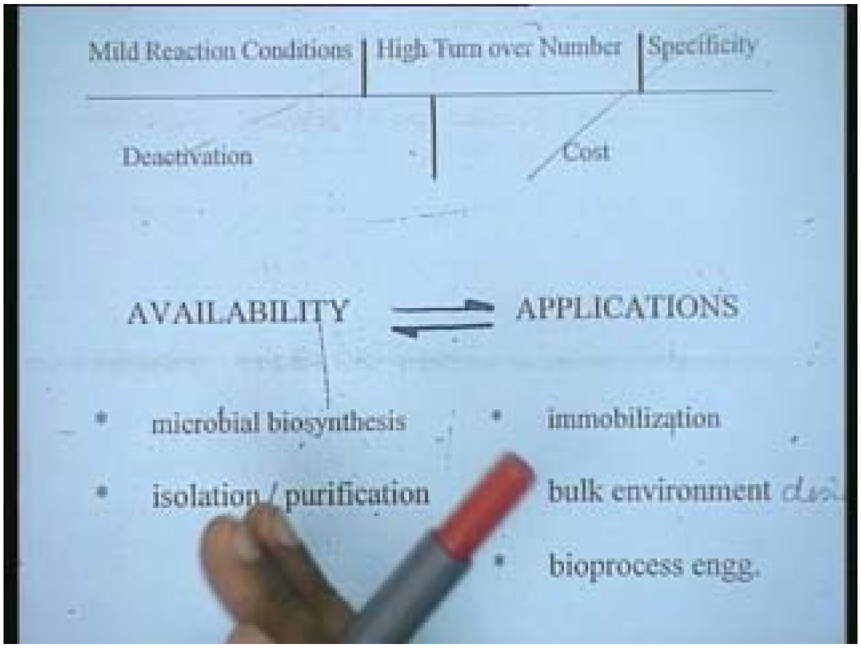

There might be some exceptions. The organisms that are found under very extreme conditions are exceptions but most of the organisms that live under ambient conditions produce or they provide us enzymes which can function under mild reaction conditions. That is the basis of their energetic efficiency. That means the energy requirement of those reactions they catalyze could be very meager.

They have high turn over number. When we discuss the reaction mechanism we will see that they are very efficient catalyst and when I say very efficient catalyst our counter part to compare is the chemical catalyst. There are a large number of reactions which can be catalyzed both by enzymes as well as by chemical catalyst and the subtle mechanisms that are involved in the enzymatic catalysis make them very efficient catalyst meaning a high turn over number or a significant reduction in the energy of activation of reaction.

The very characteristic feature of enzyme is their specificity. That means for any given reaction you need a specific enzyme and such specificity has both advantages as well as disadvantages. It is advantageous in the sense that specificity will bring in lack of any byproducts because it will not act on any of the other substrate molecule which are present as additions or contaminant in the …… stream (10.51) and you will only be reacting the desired substrate and converting it to product. On the other hand the disadvantage is from industrial point of view. You cannot produce them in very large quantity because you require small quantities of catalyst for specific reactions.

If one catalyst works on a variety of reactions the life is simple as happens in the case of chemical catalyst. For example vanadium pentoxide or colloidal platinum can act as oxidation catalyst for n number of reactions or Raney nickel for hydrogenation. Whatever has to be hydrogenated it can be used. But the same scenario doesn't happen. You need a very specific enzyme and that brings in some sort of disadvantage as far as their role as industrial catalyst is concerned.

The two of the definitely undesirable features of an enzyme as catalyst is they are relatively fragile in nature and with very mild deviations from their normal operating conditions they can be deactivated. The whole function is dependent upon a very subtle conformation. This conformation is result of not only covalent interactions but of a large number of non covalent interactions which can be disturbed very easily and that is why the deactivation of enzymes can occur and deactivation from industrial point of view is some kind of a loss.

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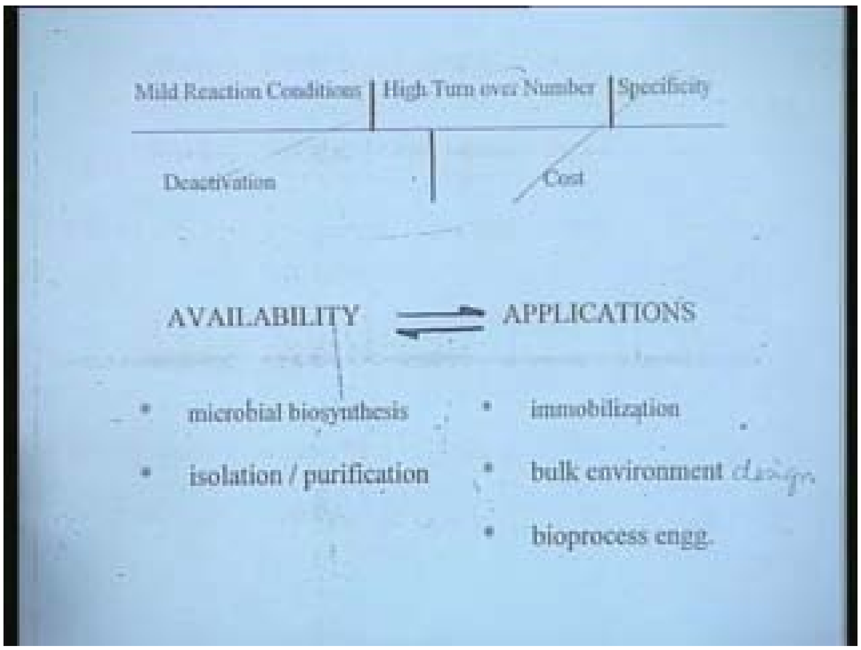

The other factor is cost which is again an undesirable feature which probably applies to any commodity we want to use for industrial purposes. We will always like to choose a commodity which is less expensive and does the job and because the enzymes are produced in small amounts by the living cell and the only source of enzyme to us are living cells and they are synthesized in very small quantities. So obviously their cost per unit quantity is comparably very high and coupled with their stability it becomes an expensive proposition. But we are also aware of the some of recent developments in biotechnology, molecular biology and other sciences which involve isolation, purification, as well as stabilization. The costs have been brought down, the concentration of the enzyme protein which can be synthesized has gone up and this situation is changing.

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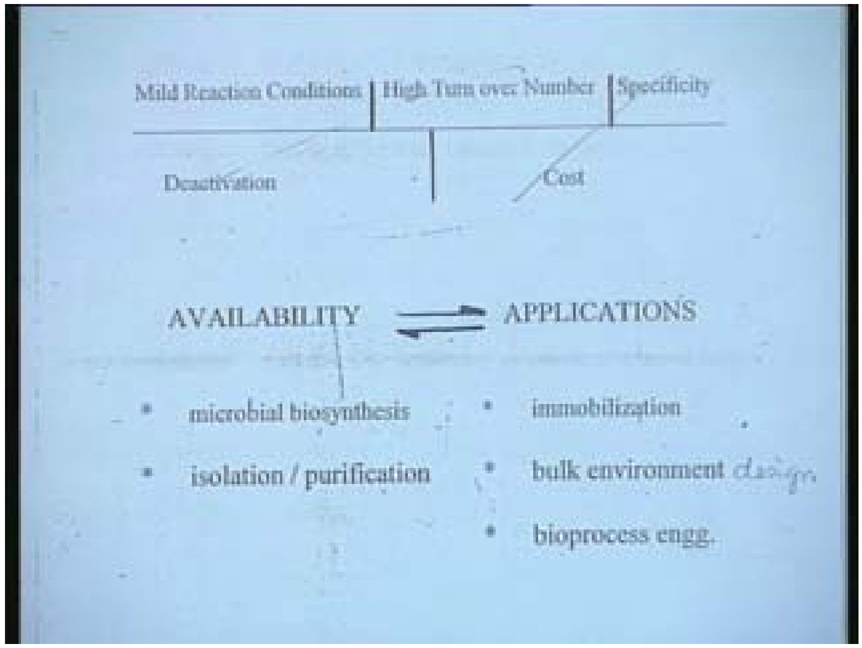

Another very significant equation as far as enzymes as processed via catalysts are concerned is between availability and applications. We saw that they carry out lot a large number of reactions. But although in a living cell various enzymes, which carry out these reactions are available their availability in large quantities for commercial applications are limited. As matter of fact only those enzymes which are required in bulk, when I say bulk I am referring to at least, if not tons, quintals many of them in tons really, they cannot be produced on a mass scale and their availability for commercial applications is limited. There are certain classical examples of amylases, glucoisomerase, cellulase, lipase, proteases, which are the enzymes that are produced in bulk and are available in large quantity. The other part is because of their bulk production the cost has also come down and become reasonable for commercial applications. The issue of availability is very intrinsically linked with the applications of enzymes. Unless applications emerge one will not like to produce them in large quantities.

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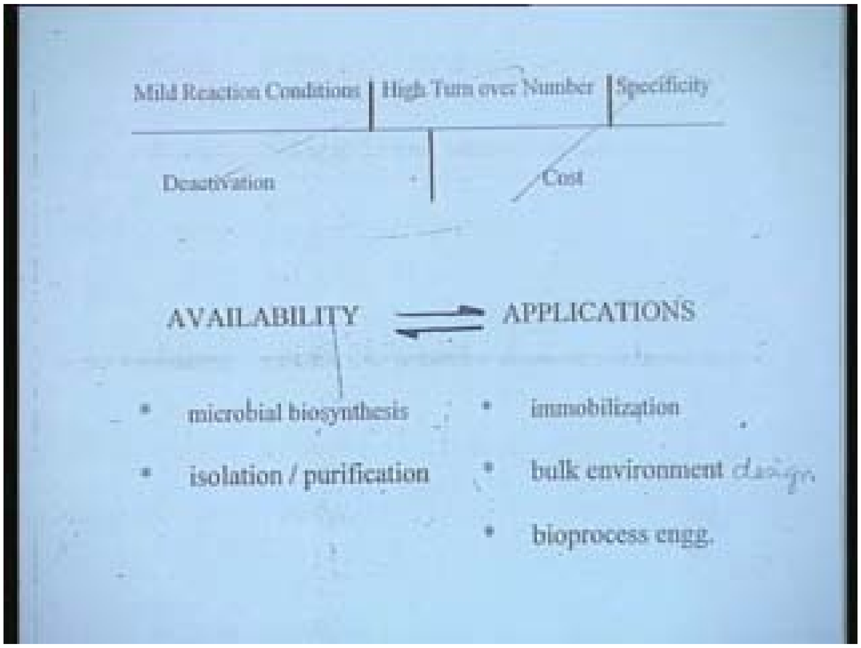

Of course the paradox remains. The applications do not emerge till they are available in large quantity. There are a large number of examples which will illustrate my point. Take for example a candidate like lipase - the enzyme that hydrolyses lipids into the consequent fatty acids and glycerol. Obviously the enzyme is not available in the quantities that many of the other enzymes, like amylases and proteases, are available. But there is a potential that it can be produced provided the applications emerge so that the scale of operations which is required for bulk operation can be easily maintained and so the reversible interaction between availability and application is a very intrinsic feature and probably will illustrate the story of many of the enzymes which have become commercially viable today. As far as availability is concerned there are two major factors. One is that we have to synthesize them.

Theoretically one can produce enzymes from any living source microbial, plant or animals but the trend in the reason past has been that the microorganism has been the preferred choice for variety of reasons. Some of you may be familiar with them and most important one is diversity. Secondly you can choose micro organism which will be able to act on a particular substrate, synthesize required enzyme. On the other hand you also have a very clear understanding of the genetic machinery of the cell, the microbial cell that controls the enzyme synthesis, which you will manipulate leading to hyper production of the enzyme.

Their bulk productions, their bulk growth is very simple. The parameters, the equipment are all standardized thanks to many other products which are produced microbially that gives an edge for microbial biosynthesis of enzymes. The other important part is isolation, purification and stability.

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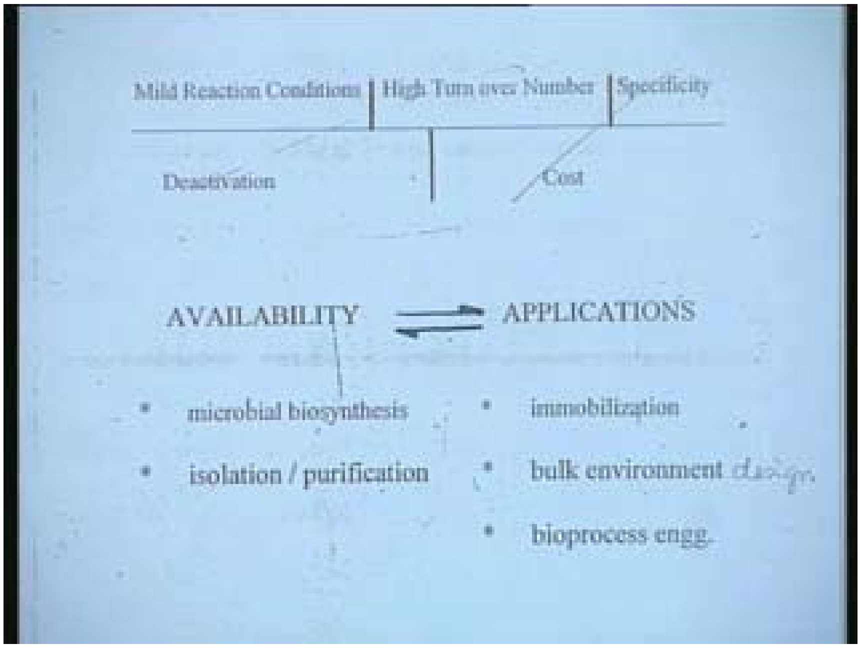

In isolation and purification one of the very characteristic features is that most of the enzymes are purified by a series of steps. An electrophoretically homogeneous protein means it is a perfectly pure enzyme.

For most of the industrial applications except for some of the medical or diagnostic applications you will not require a highly purified enzyme. For bulk industrial processing we have to stop at purity level some where in between. Compared to this crude enzyme where this specific activity is very low to a level where the contaminating enzyme that can disturb the process are revoked. But note that all the contaminants are revoked which may not be required because any order of purification will mean additional cost, enhancement in the value of the product.

As far as the applications are concerned a lot of engineering inputs are required particularly for the extended use of the catalyst and we don't want to use it just once. As a matter of fact I think one can take a clue again here from a living cell. The cell maintains a very perfect economy in terms of its energy as well as materials to synthesize proteins. It doesn't use the proteins only once. It uses in a cycle and over a period of time. There is always a maintenance part where it has to maintain the protein synthesis but it is being used repeatedly and in many of the organs of the cell, the enzymes are supported on certain organelles so that they behave almost in a fashion of insolublised enzymes.

On the basis of the same concept one can immobilize the enzymes on a support, insoluble support what we call as immobilize enzyme and use them for processes that can be used in continuous mode like any other chemical process, the term very commonly used in the chemical industry is heterogeneous catalysis.

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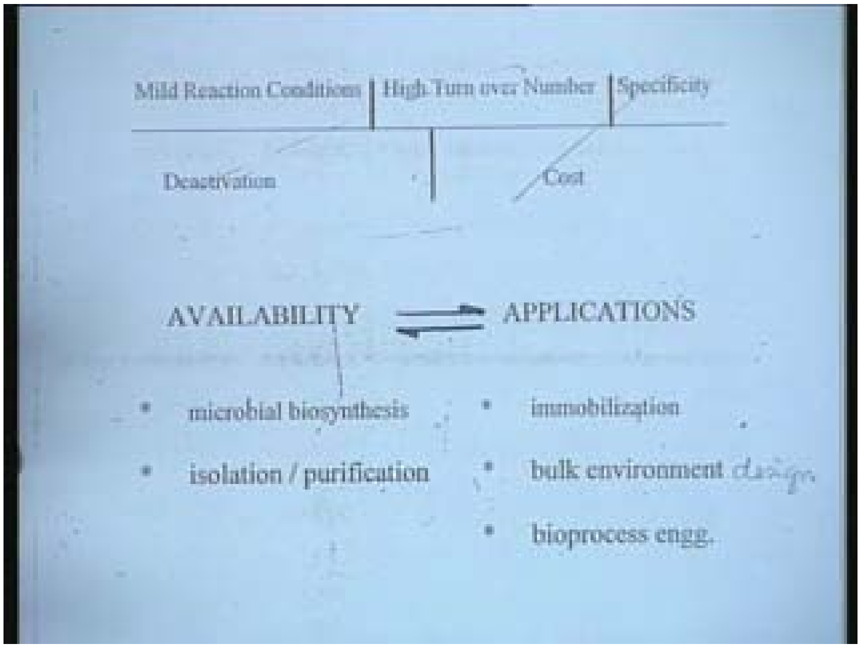

Then a very important aspect is bulk environment design. So far we have been limited in our mind regarding the application of enzymes that they can work only in aqueous environment. This today is probably not a very perfect statement because a number of enzymes have been shown to function even in a non aqueous environment or in other words more accurately micro aqueous environment where you require a very small concentration of water, rest of the bulk environment will be replaced by an organic solvent and that can give us a whole new range of chemical reactions which can be carried out using the enzymes and biocatalyst particularly a transition from hydrolysis to synthesis and many of these reactions have become commercially viable today.

Ultimately for any process application of the enzymes you need to engineer the systems even after you have done immobilization, even after you have studied the optimum reaction parameter, the whole systems has to be engineered. I mean to say that you have to develop particular hardware reactor, an enzyme reactor in which the whole reactions can be carried out with maximum productivity because ultimately the whole concept or the whole interest between will be on the productivity and which can be tailored by doing the proper process engineering. Then you may need to monitor certain parameters, the instrumentation control, down stream processing of the product, all has to be integrated so as to have a complete application route.

In summary the broad objective of this course as I mentioned so far will be to study various scientific, technological and engineering aspects associated with the application of enzymes as a bio catalyst in process industry.

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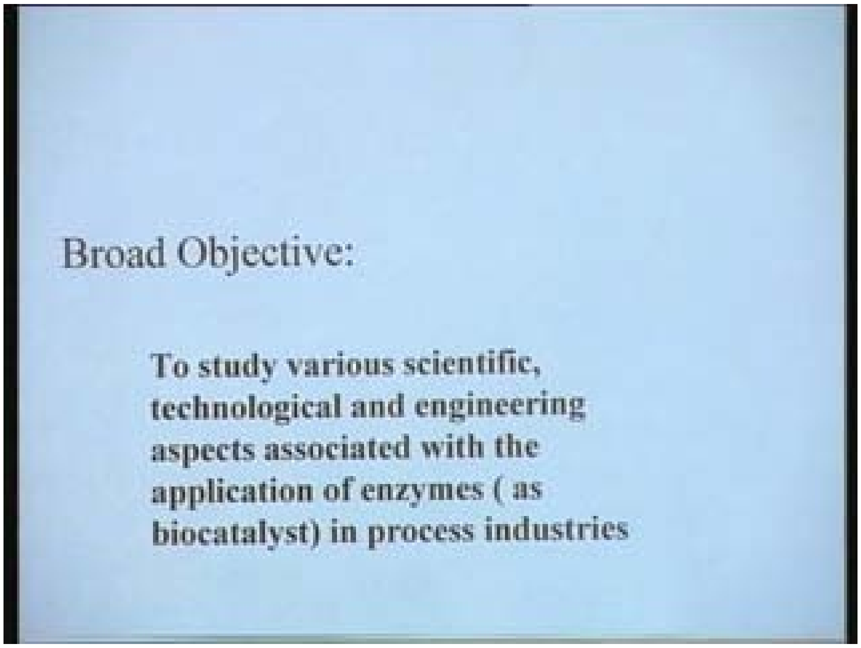

This in brief terms can be the broad definition of or broad objective of this course. I like you to be very critical about the scientific, technological and engineering aspects. Almost an analogy with the biotechnology in general even the enzyme technology also has a very multidisciplinary nature. It is not that one discipline for example understanding only biochemistry can lead to understanding of the applications of enzymes in the process industries. A whole range of basic and engineering sciences are required to be understood. For example if you start let us say a basic science like chemistry. Many aspects of chemistry have to be understood in relation to enzymes or in other words you can add the term biochemistry. The functional aspect of the enzymes like the kind of chemical reactions they catalyze, the thermodynamic limits, the equilibrium convergence, the free energy change, whether the reaction is feasible, the coupling of reactions so that they become feasible in terms of free energy change and all those aspects of the enzymes protein will come under realm of bio chemistry.

A very important role is played by physics. Today a large variety of developments that have taken place in the area of biotechnology in general and enzymes in particular can be attributed to the use of a number of physical techniques which have made us to understand a variety of systems and develop and improve upon them for various applications and physics becomes a very important component to understand the enzyme role. You are familiar that the result of these applications and developments is the science of biophysics as you know it today. After chemistry and physics I think the major scientific component will be biology.

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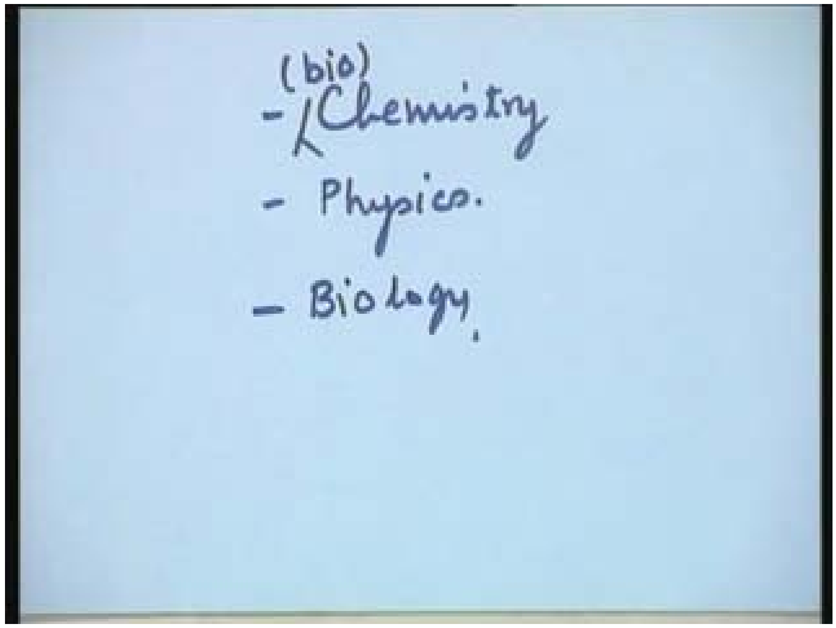

For any enzyme to be produced in large quantity you need to identify a biological system which can produce the enzyme. It can be microorganism, it can be a plant or it can be animal. The bulk of the applications or in the bulk of the cases of enzymes we will look at microbial systems. That is the component which biology will make us understand. We will have to understand its morphology, its physiology so as to be able to design an appropriate enzyme production system.

The role of protein biosynthesis were their (25.20) organism which will regulate the synthesis of enzymes. The regulatory mechanisms involved in the bio synthesis of those enzyme proteins will also be understood through biology. In the recent years an added dimension is molecular biology where we tend to understand the structure function relationship of various biological molecules at a molecular level. How nucleic acids take part in information transformation from one cell to next generation, what are the mechanism of cell-cell signaling, communication between different cells or how does the protein molecules behave as a defense molecules? All variety of those functions at a molecular level has become an added dimension which has been at the center of the bio technology in general. Similar applications will come here for biology also. A very important scientific development has been in the computational science.

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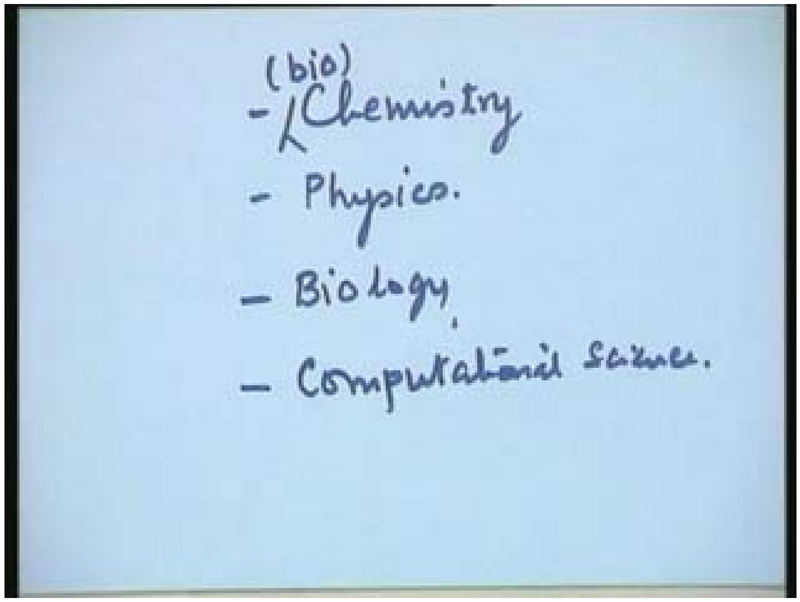

Most of the industrial applications will require the application of computational science in those systems. Today even design of an enzyme protein, modifications for tailoring it to give some desirable property is a job which can be taken up only with the help of computational science. Experimental sciences will take years together to come out with those conclusions which can be brought in by many of the computational programs. You will need lot of data available to you before you can apply them but once the data are available one can easily compute and predict many of the behavior which otherwise would have taken a very long time. For example site directed mutagenesis is what happens if an amino acid is removed and another amino acid is inserted in the polymeric chain that is in the protein chain. Its structural behavior can be very easily understood with the computer graphics which otherwise doing experimentally probably is a Herculean task. These are some of the basic sciences which are important.

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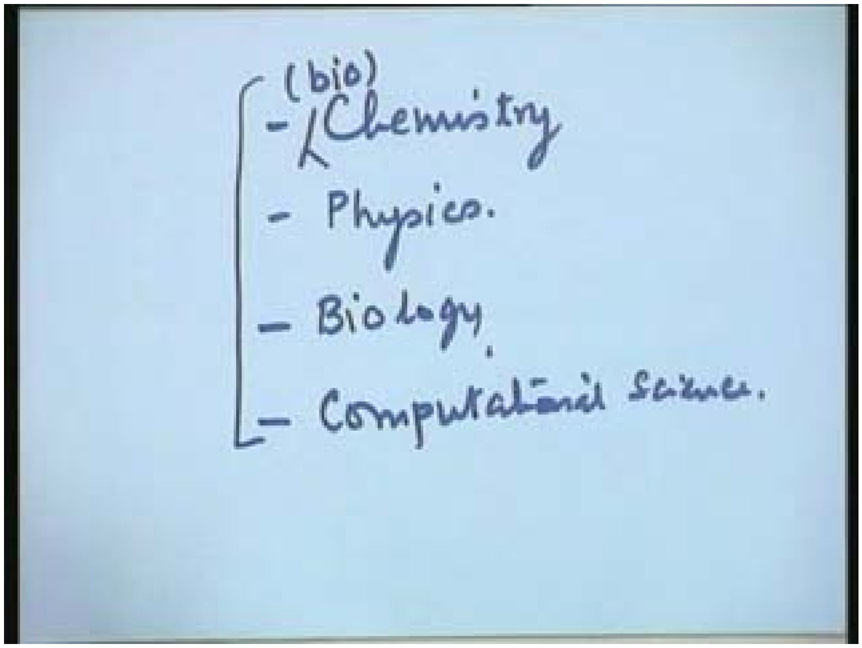

On the other hand we have a whole range of engineering sciences which are important as far as enzyme technology is concerned. You need to design enzyme reactors which can take care of transfer of materials; transport of energy, transport of momentum and all transport processes can be taken care. You need instrumentation for monitoring and control of the reactor, you need the concept of electronics particularly in some applications like biosensors, enzyme will require transducers and the ultimate is biochips. We are far from the reality but still as the concept it has probably attracted the attention of many scientists. That means can be used in the protein molecule or enzyme molecules as biochips in the computers.

So the whole computation becomes based on biological molecules. So a large number of engineering operations including mass transfer, heat transfer, material balance, energy balance, product separation, kinetics, thermodynamics is also involved in understanding of enzyme science and therefore one of the characteristic feature is that the whole enzyme science interfaces with a large number of basic engineering sciences and it is this interface which makes it so attractive to study, exciting to study and important to study. Today it has become impossible probably for a bio technologist to leave aside an enzyme molecule, understanding of enzyme molecule in detail before he can call himself to be a professional in the area.

Another important aspect which I think I will like to touch upon in today's lecture will be on enzyme classification. Now you will be surprised to know that so far globally about three thousand enzymes have been isolated, characterized and reported in literature. It's not a very large number compared to the feasibility of combinations that are feasible. If we look at enzymes as a polymer consisting of nineteen different amino acids with an average length, let us say 150 or 200 amino acids linked together. With nineteen different amino acids probably the possibility of, variety of, diversity of proteins that are feasible is enormous almost approaching infinity. We have been able to isolate and characterize only about three thousand. Now this three thousand, just to understand and bring it into a systematic feature, have been classified into six different classes. In fact this job was done way back in early seventies by international union of pure and applied chemistry, what you know as IUPAC. They gave a classification system which is acceptable globally today and it classifies all the enzymes into six major classes': oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

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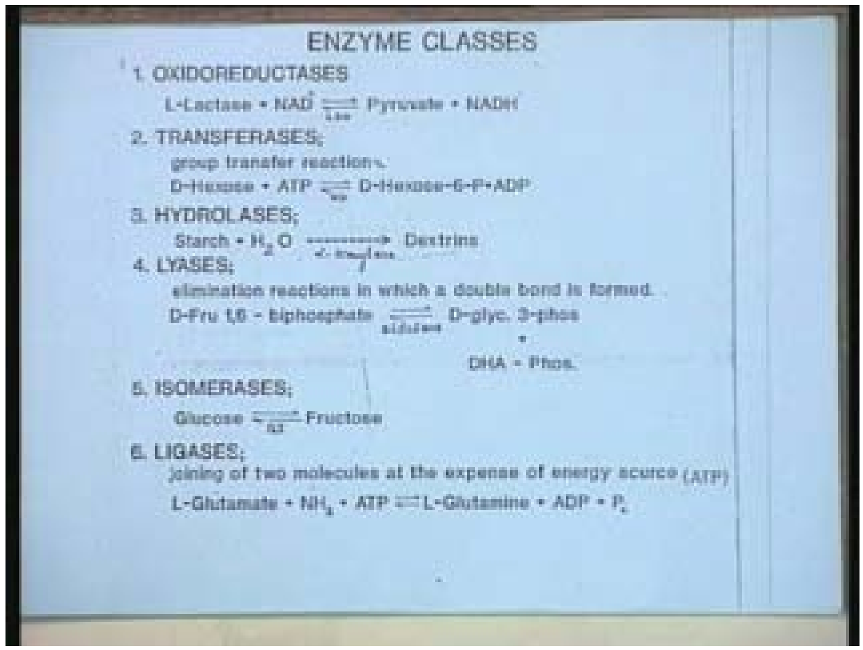

The whole range of chemical reactions or bio chemical reactions that take place in a living cell are mediated by these different classes. We must know that some of the reactions are not independent. They are coupled there by producing effect which could be a polymerization reaction. Directly a polymerization doesn't take place as it is. It is mediated by series of transferases and then ultimately the transferases and ligases. Ultimately you end up with the polymer of different monomers. But basic enzymes can be classified and each of this class is also characterized in sub classes. Sub classes refer to the type of substrate or the type of the product that is produced by those enzymes. So basically the whole range of each of the class here oxidoreductases a bulk of the oxidases, reductases and the hydrolases fall under this category.

Transferases involve group transfer reactions where one of the functional group with a substrate molecule is phosphorylated. Usually they are bisubstrate reactions involving two substrates. In hydrolases by and large the most commonly employed industrial catalyst is used mainly because of simplicity. Because the co-substrate is water which is present in large quantity and one can use them for hydrolytic reactions. By changing the bulk environment hydrolysis can be tailored to be used for synthetic reactions as well. In lyases an additive enzyme which catalyses the elimination reactions that result in the double bond formation is used. Isomerases are another important catalyst of industrial enzymes which isomerises one molecule into other isomerable molecule. One of the most significant enzymes under this class is isomerization of glucose to fructose. Ligases join two molecules at the expense of energy source. In most biochemical reactions ATP is the energy currency which is consumed and regenerated through catabolic processes and such reactions are classified under ligases.

This is the classification based on the IUPAC nomenclature more from the scientific point of view. But from the industrial point of view or from the application point of view another important type of classification which is made is in terms of three classes what we know as bulk, diagnostic and therapeutic.

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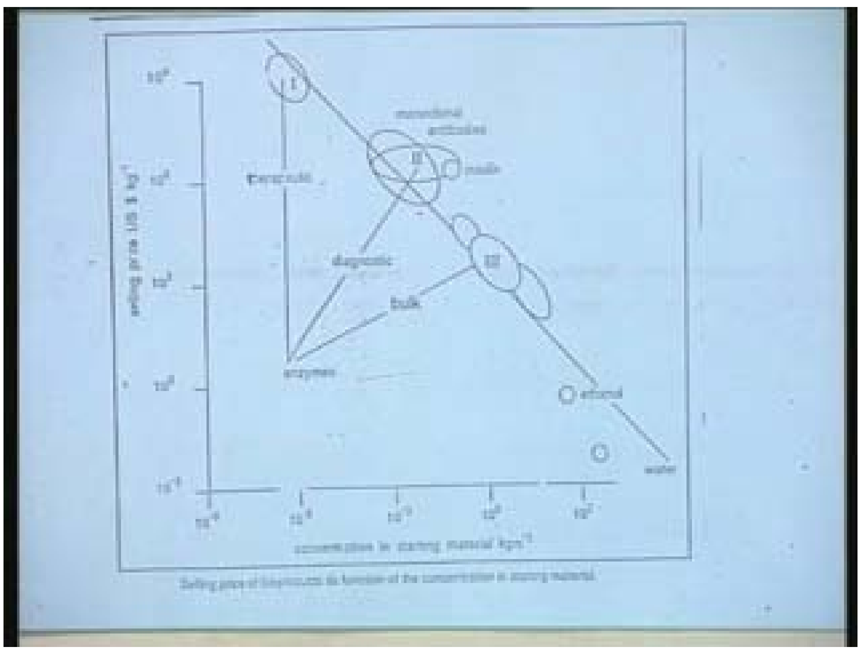

This classification is based on the cost of the enzymes. In fact the cost of the enzyme is directly linked with the concentrations in the starting material. It could be a fermentation broth in most cases. In the fermentation broth if the concentration of the product or the desired enzymes is very low the cost is very high and second factor of cost is the application and the degree of purity you need for that particular application. The bulk enzymes as you can see here they fall in this category. The concentration in the starting material is of the order of about one kg per meter cube which is significant enough to recover and you don’t require for the most cases a very high degree of purity and therefore their cost is also reasonably low say for example about thousand US dollars per kg.

The next category is diagnostic. It includes also analytical enzymes. This is the concentration in grams per meter cube level which is much lower. Degree of purification required is also larger because you will not like any protein contaminant present in the sample and their cost is therefore significantly high of the order of about 10^6 US dollars per kg.

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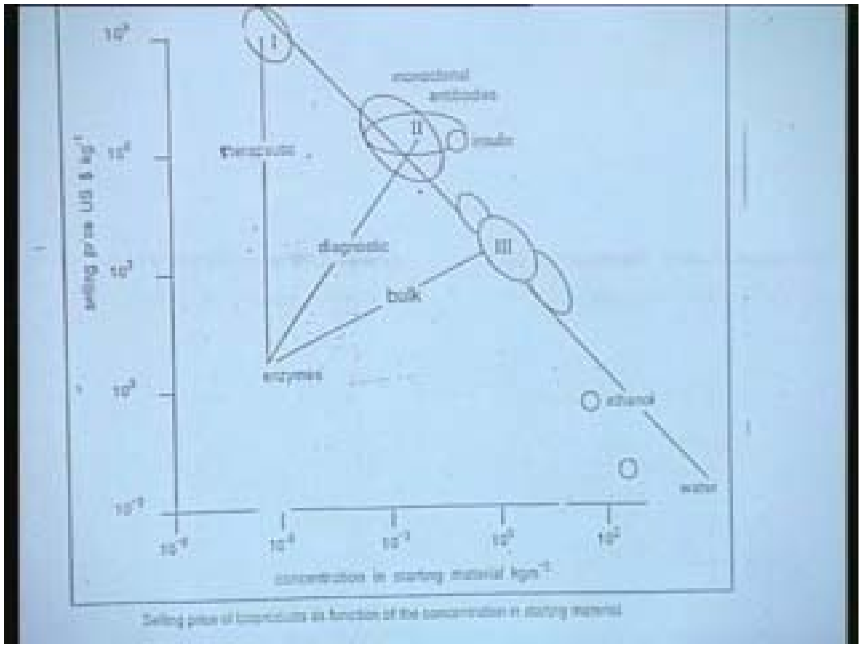

You should not get threatened by the amount of cost of 10^6dollars. The quantity required is also in micrograms. They are not in kilograms like other molecules. So they are affordable.

The third and probably most expensive classes are therapeutic enzymes which are exclusively required for medical applications almost like life saving drugs. Their cost is immaterial and you don't make choice on the basis of cost. In the case of industrial operations, you always make a choice between two alternatives on the basis of the cost and effectiveness. But if effectiveness is the same the cost is the principle determining factor. In the case of therapeutic products, the choice is not on the basis of cost but on the basis of its need if it is a life saving drug. Many of the therapeutic enzymes particularly the recombinant proteins that are available from the enzymes like streptokinase and urokinase are blood clot dissolving enzymes. Within fraction of a minute they simplify the situation of a cardiac failure problem due to blood clot. Similarly a number of other such therapeutic proteins or enzymes are available like asparaginase, for patients suffering from leukemia, which is again life saving. The cost is also of a very high order but the quantity required is of less than micro gram quantities but in a very highly purified state.

The last phase of today's lecture will cover on course outline and references and the course will broadly cover these aspects. I have given you some idea about the introductory remarks on the enzyme as process biocatalyst. We will go into little more details on the chemical nature of enzymes that means enzymes or proteins. We will go into functional nature of enzymes, enzymes as the catalyst and what makes them such an efficient catalyst? The factors which are responsible for their deactivation, the approaches that are available for stabilizing the enzymes, the role of active sites or ligand binding sites on the enzymes will be dealt in functional nature.

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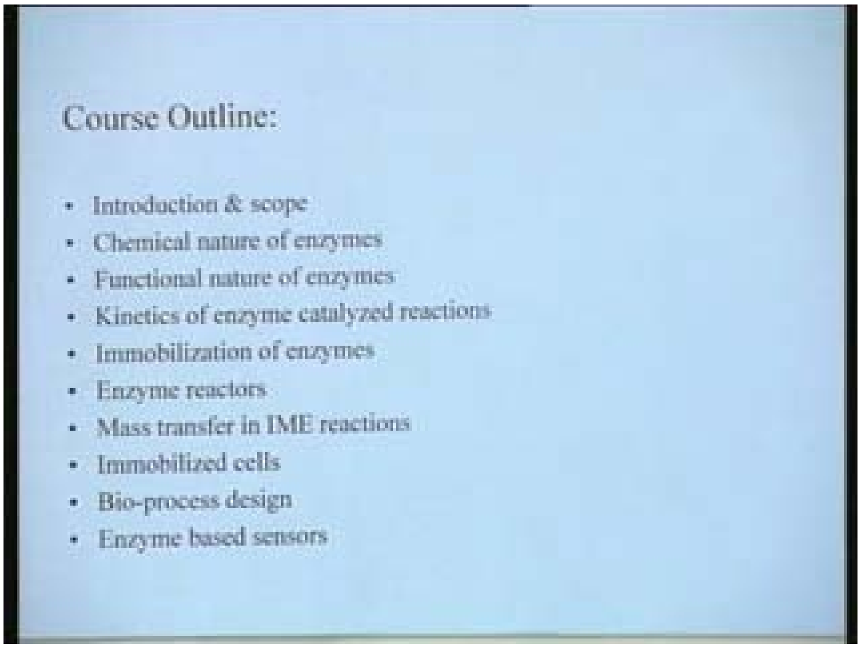

Then we will go on to the kinetics of enzyme catalyzed reactions because reaction kinetics will play a very important role in the choice of enzyme reactors, to understand reaction rates and the role of associated physico-chemical interactions that will take place once you immobilize the enzyme or use them in bulk environment other than water. One of the most significant tools which have made many of the enzyme reactions commercially viable is immobilization of enzymes.

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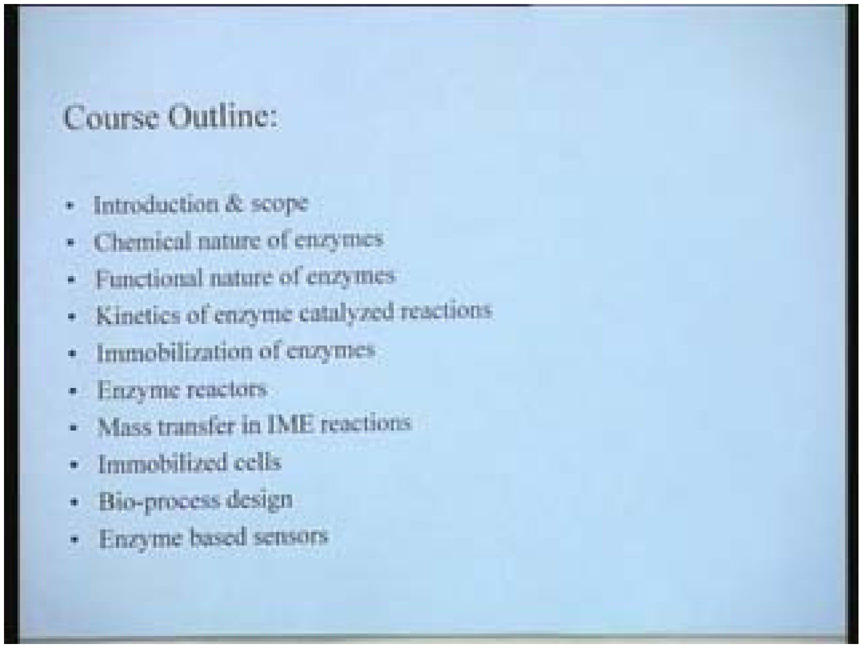

A large number of techniques are used and we will discuss them in detail. The use of enzyme reactors, the types of reactors that are possible to be used with the mobilize enzyme, with soluble enzyme, their characteristic features, comparison between their performances, how to choose the enzyme reactors, then mass transfer and immobilize enzyme reactors. Immobilization of enzyme will have some additional physico chemical features like diffusion and partitioning.

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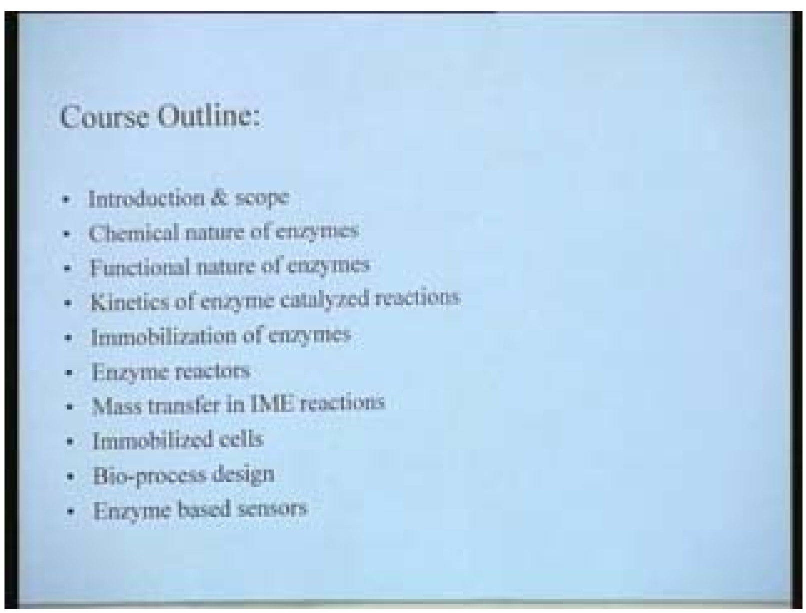

When they are placed in a reactor how do they influence the performance of the system? Immobilize cells very often in the enzymes are intercellular. It has been felt that for many applications their isolation and purification is not required. You can immobilize the whole cell as long as you do not inactivate other contaminating enzymes in the cell which will interfere in the process.

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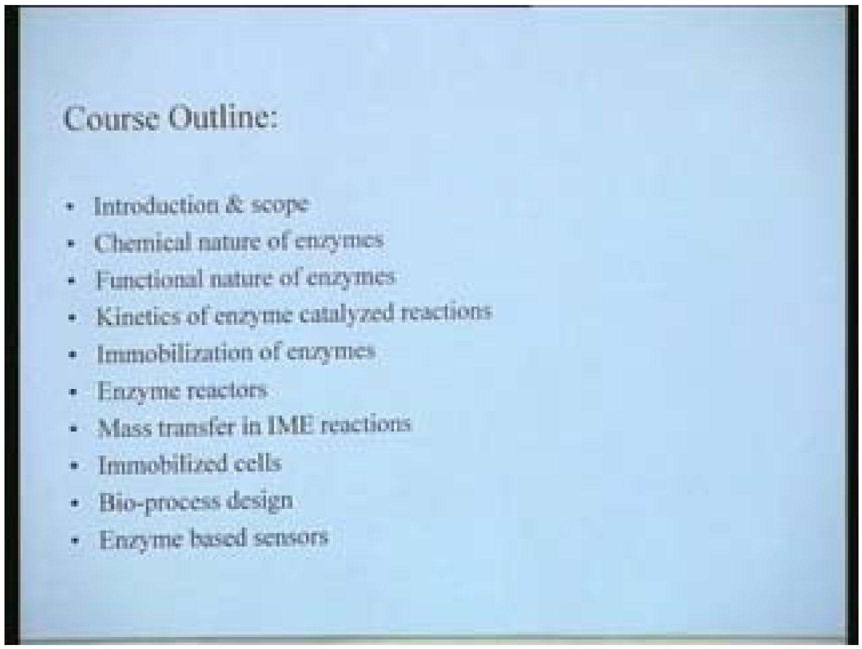

In the bio process design, some of the basic design principles for the enzyme catalyzed processes. A few case studies I like to illustrate some of the earlier issues with respective to specific cases and ultimately I think I like to take up enzyme based sensors. The enzyme sensors have become an important activity where immobilization enzymes are used. Both immobilized enzymes and immobilized cells are used. They have become very important tool both in diagnostics as well as monitoring of information from reactions. This will be our course outline.