Biochemical Engineering

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Module No. # 01
Lecture No. # 08
Biochemistry and Thermodynamics of Enzymes

This graduate level course on Biochemical Engineering.

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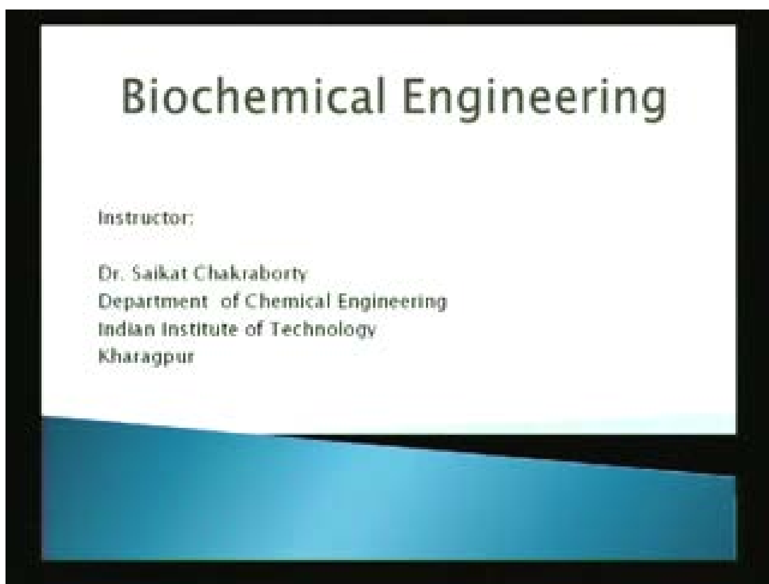

And this is the course, what we will try and do is give you a sense of the coupling between biochemistry and transport and reaction as applied to chemical engineering.

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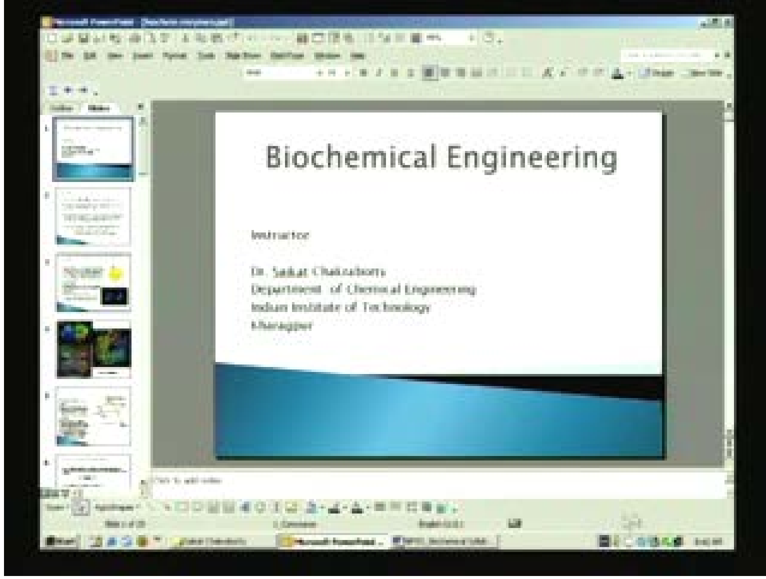

So, before I am going to the details of today's lecture what I try and do is, give you a sense of the overall outline for the course.

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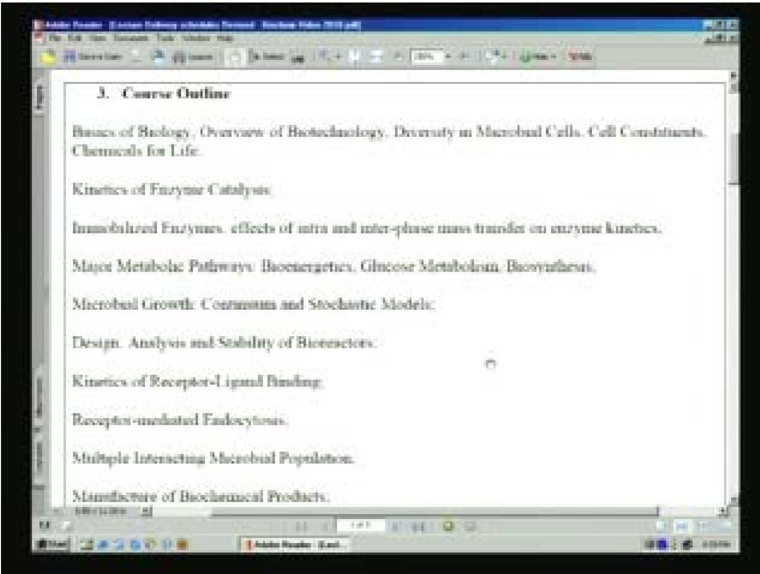

3. **Course Outline**

Basics of Biology, Overview of Biotechnology, Diversity in Microbial Cells, Cell Constituents, Chemicals for Life.

Kinetics of Enzyme Catalysis.

Immobilized Enzymes, effects of intra and inter-phase mass transfer on enzyme kinetics.

Major Metabolic Pathways: Bioenergetics, Glucose Metabolism, Biosynthesis.

Microbial Growth: Continuum and Stochastic Models.

Design, Analysis and Stability of Bioreactors.

Kinetics of Receptor-Ligand Binding.

Receptor-mediated Endocytosis.

Multiple Interacting Microbial Population.

Manufacture of Biochemical Products.

Let me try and make this larger, so this is this is some of this things that, we are going to cover for example, basic of biology, overview of biotechnology, diversity in microbial cells, cell constituents and so on. So, essentially to give you an idea of you know, how cells works and in the earlier course that, we did transport processes we had a sense of how cells work and what are the you know essential ingredient of the cells, the cell

membrane and so on. In this course, so what is it major difference between in the early course and this courses is that, there we we concentrated little more on the transport phenomena, here the emphasis this more reaction engineering aspect of it, but of course, you cannot deal with reaction engineering separated from transport phenomena.

So, we are going to look at the coupling between the two and the biochemical the whole series of biochemical reaction that happen, how cells respond to it that is at the cellular level; and as always our emphasis is on, how to move from one scale of analysis to another. So, we can move on from the cellular level, so that is microscale to the mesoscale to the macroscale. So, if you look at this, so at this cellular level for example, we start the course essentially at the cellular level, where we talk about basic of biology and so on.

And the kinetics of enzyme catalysis, which is you know the the chapter that we are going to start today. And then, we look at something like immobilized enzymes and I will talk about immobilized enzymes, when we come to get little bit and talk about metabolic pathway and so on and microbial growth model. Then, we go to the largest scale, where we deal with, when we do deal with the microbial growth, the large scale of where you talk of not just one cell, but a cluster of cells and how these cells grow. So, now you you are moving on from the microscale, which is the scale of single cell to the to the macroscale, which is the mesoscale sorry, which is the scale of a cluster of cells and so on.

So, and then the next step we go on to design analysis and stability of bioreactors, which is obviously, the microscale right. So, now we look at not just the reactor design, but when you use these biochemical reactions, when these biochemical reactions happen in the reactor, what kind of dynamics it generates and these are pretty interesting dynamics and different from the kind of dynamics that, you would see elsewhere.

When it comes to microbial growth for example, we will we will look at some of the growth models stochastic and the continue models of microbial growth and why this is important? Because, you know when you are doing cell culture in the lab right, so some of you might be doing the cell culture in the lab, when you are doing that it is important that you figure out, how cells actually grow and we what we learn we will do some

models of cell growth and try and understand, how cells actually grow and then, apply those to the biological reactor, the bioreactor you know the chemostat.

One of the important things and interesting things actually that, we are going to do is kinetics of receptor-ligand binding. So, what is receptor-ligand binding? It turns out that, most of the physiological processes, you know we did some of the transport things before, but the reaction part of it most of the many of important physiological processes are dictator are govern by reactor by reactor ligand binding.

So, they are dictator that are govern by reactor ligand binding that, the so the reaction between the reactor and ligand is necessary ingredient and some of you actually are working on that. So, this is a necessary component of not just how cells work or function in day to day life, but also in diseases and and therapy of diseases also.

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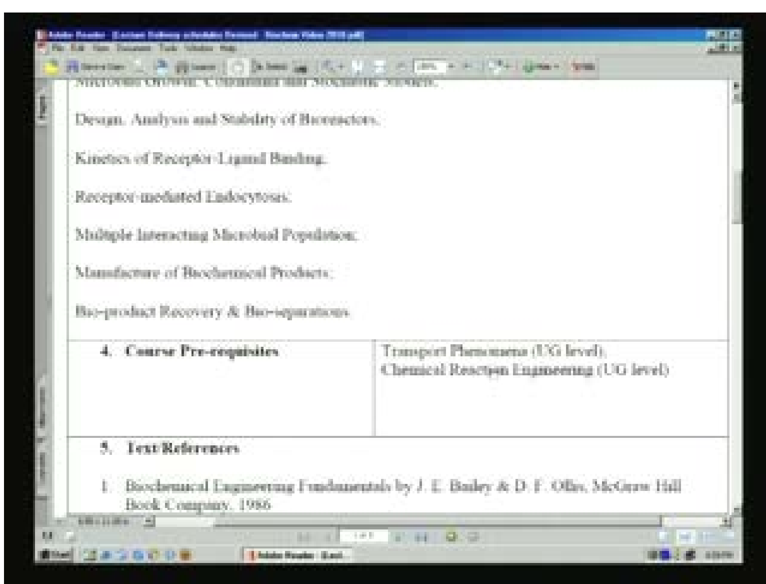

And little bit of regulation of gene expression and bio-product recovery, which professor (( )) is going to do.

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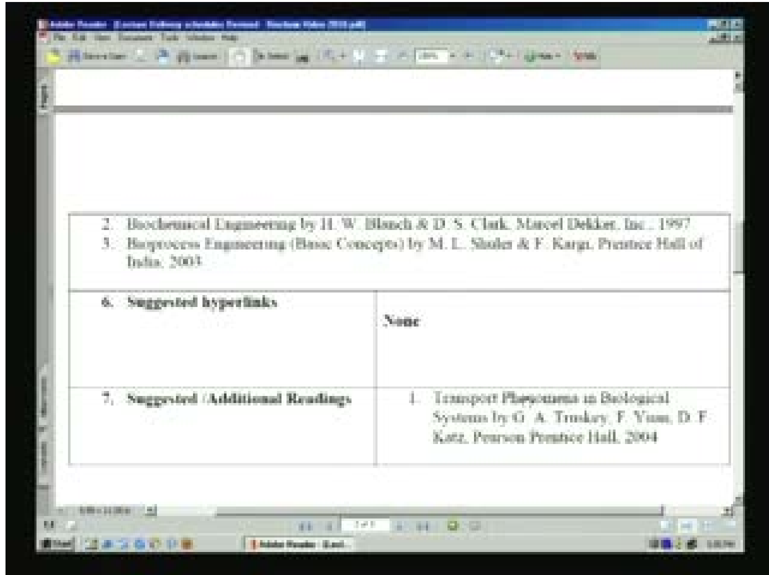

You know let me go through the books that are involved and you know that, might want to look at in this course. So, the first book is the the most important book, biochemical engineering fundamentals by J. E. Bailey and D. F. Ollis, this is one of the most classical books of you know.

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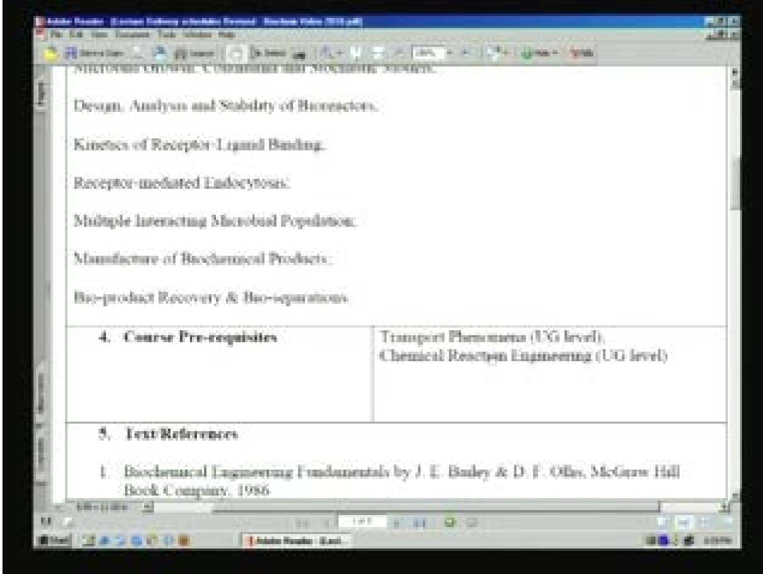

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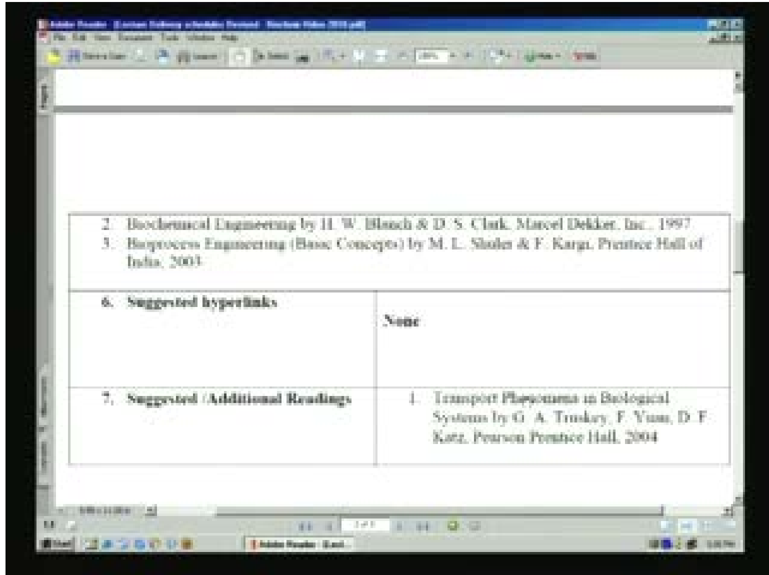

2. Biochemical Engineering by H. W. Blanch & D. S. Clark, Marcel Dekker, Inc., 1997 3. Bioprocess Engineering (Basic Concepts) by M. L. Shuler & F. Kargi, Prentice Hall of India, 2003	
6. **Suggested hyperlinks**	**None**
7. **Suggested /Additional Readings**	1. Transport Phenomena in Biological Systems by G. A. Truskey, F. Yuan, D. F. Katz, Pearson Prentice Hall, 2004

So, this is one of the most classical books of chemical biochemical reaction engineering, Bailey used to be faculty actually in the graduate school, I went to and he is he is one of the most well known people in biochemical engineering.

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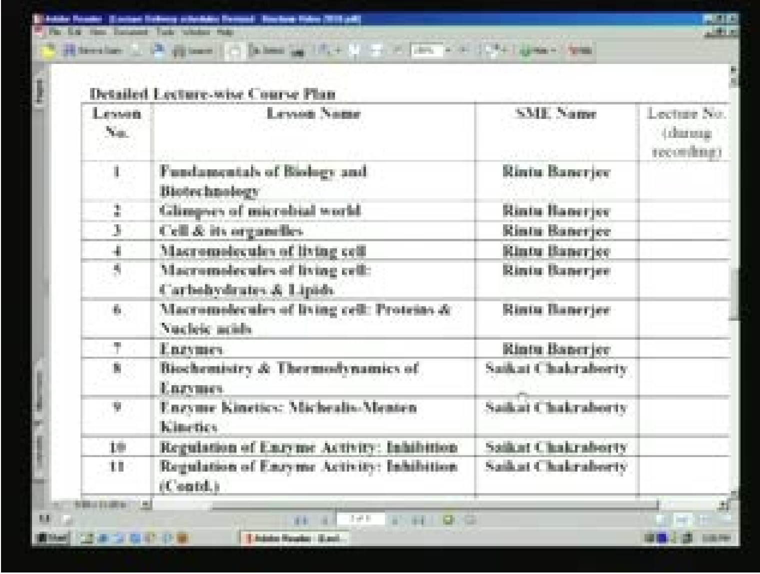

Detailed Lecture-wise Course Plan

Lesson No.	Lesson Name	SME Name	Lecture No. (during recording)
1	Fundamentals of Biology and Biotechnology	Rintu Banerjee	
2	Glimpses of microbial world	Rintu Banerjee	
3	Cell & its organelles	Rintu Banerjee	
4	Macromolecules of living cell	Rintu Banerjee	
5	Macromolecules of living cell: Carbohydrates & Lipids	Rintu Banerjee	
6	Macromolecules of living cell: Proteins & Nucleic acids	Rintu Banerjee	
7	Enzymes	Rintu Banerjee	
8	Biochemistry & Thermodynamics of Enzymes	Saikat Chakraborty	
9	Enzyme Kinetics: Michealis-Menten Kinetics	Saikat Chakraborty	
10	Regulation of Enzyme Activity: Inhibition	Saikat Chakraborty	
11	Regulation of Enzyme Activity: Inhibition (Contd.)	Saikat Chakraborty	

So, if you want to buy any book and if you you know if you are interested in buying any book in biochemical, this would be the book to buy and look for references, the other important books also are you know by Blanch and Clark and this the Clark from Berkeley and bioprocess engineering by Shuler and Kargi that is also nice book as well.

The last book that I am going to just teach one chapter from this book the suggested reading that is there transport phenomena in biological systems by Truskey, Yuan and Katz and this pretty expensive book. So, I do not suggest to buying that and I will give the notes you know we will do do things in the classes. So, we can take notes, but you know if you are interested you can buy that as well. So, these are the four books and between these four books you know everything that we will do in this class are there between these four books.

So, let me now now give you a sense because, this is the lecture by lecture things. So, let me just give you a sense of some of the lectures that we are going to do, two people teaching that this course. So, I just want to show you how they split up. So, I am going to teach the basic of biology and the overview of biotechnology and microbial cells and constituents of them. I am going to start today with biochemistry and thermodynamics of enzymes and the next class tomorrow; we will do the kinetics of enzyme catalysis.

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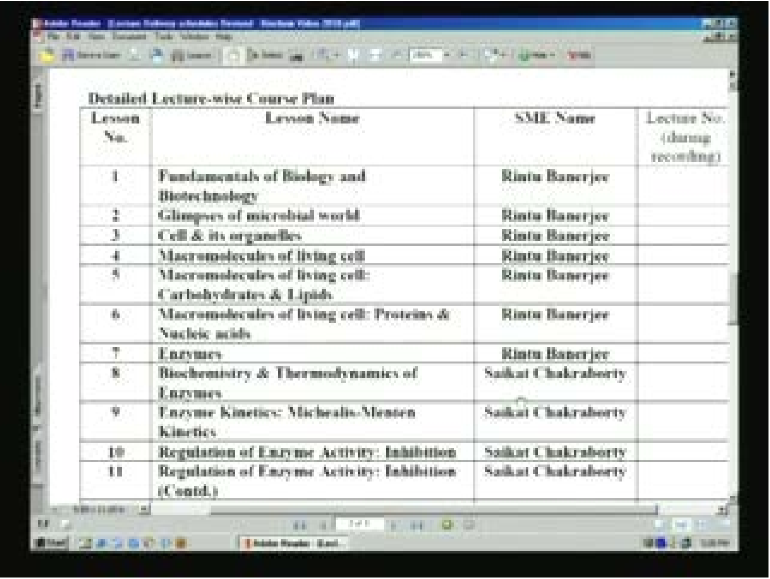

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And follow by regulation of enzyme activity, comparative, non-comparative and inhibition and so on. Then, we move on to immobilized enzymes, hydrogenase enzymes catalysis, what are immobilized enzymes you know enzymes to start with let me give you very quick wrap on this enzymes to start with are catalyst right, these are essentially catalyst that catalyze biochemical reaction.

So, in normally in chemical reaction engineering or chemical reaction engineering you have catalyst that catalyze normal chemical reactions here they just catalyze the biochemical reaction. So, that is major fundamental different how they different, what can you think of what are immobilize enzyme, what could be immobilized enzyme, because you know one of things the hint that is there in number 10 is the immobile enzyme immobilization, hydrogenase enzyme catalysis. So, what can you.

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No.

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Right. So, you know the enzymes that we talked of initially are essentially liquid phase enzymes that they are in the liquid phase and then, they dissolves or or soluble in the liquid and then they go and the substrates comes to them and they help in reaction and so on. Immobilized enzyme is the name suggesting is very straight forward and enzyme which are not moved, which do not move. So, how do you how do you achieve that, what you do is you kind of restrict them to matrix and I will I will show details of that you know when when you go to that we will kind of restrict them to matrices certain matrices kind of interact them in certain matrices and why these immobilized enzyme important.

See, I will give simple example when you have for example, you know in indigestion you just ate something heavy and you want want to get some some some digestive medicines. So, what are these digestive medicines? There enzymes right, these are gelusil or carmozyme or whatever, these these are essentially enzymes, which help in the digestion process. So, there are two kinds of these medicines that you take some are liquid medicines and some are tablets right, so that is example, so liquid medicines that you have normal enzymes in the liquid phase, which go on you know they work faster a little bit and tablets are immobilized enzymes.

So, you have this you know all kinds of tablets pudin hara, gelusil all kind of these. So, these are essentially immobilized enzymes, enzymes in encapsulated form, it will take little bit of time to time to dissolve into the system and to work little bit more than the liquid phase enzymes, but the advantage of these immobilized enzymes are that, you can

take them along with you right wherever you go they mobile. So, though though they called mobilized this is something that you can take them along with you if you want. So, we have to understand the the you know kinetics of that and the the coupling between the kinetics and transport of that, which is lot more complicated than normal simple enzymes, liquid phase enzymes why is that, why is that its answer is right there in front of you.

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Yeah because, these are hydrogenase system, so when we talking of liquid phase enzymes, these are homogenous system where we have been talking of solid phase enzymes are in mobilized form, these are hydrogenase systems and therefore, you have to understand the hydrogenase catalysis and coupling between hydrogenase catalysis and reaction, which is little more complicated thing. I am not sure if you have study have been taught that in reaction engineering probably not. So, we will go in to the details of it little more.

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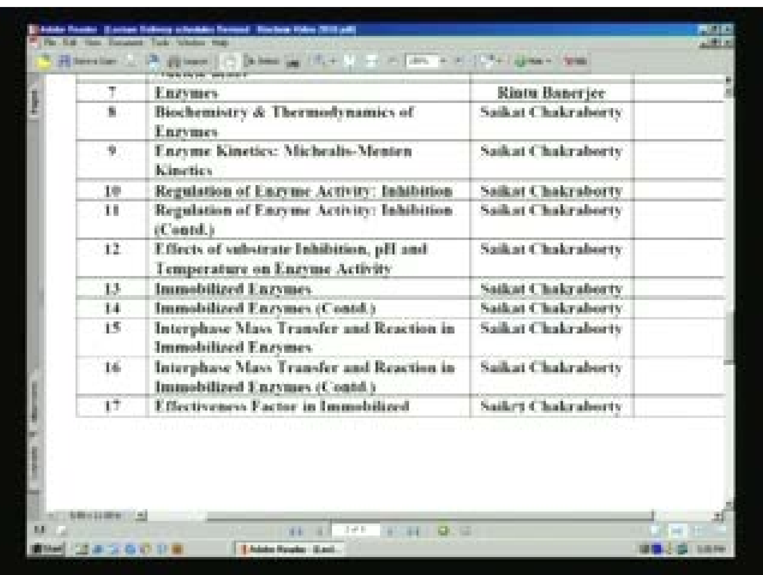

7	Enzymes	Rintu Banerjee
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12	Effects of substrate Inhibition, pH and Temperature on Enzyme Activity	Saikat Chakraborty
13	Immobilized Enzymes	Saikat Chakraborty
14	Immobilized Enzymes (Contd.)	Saikat Chakraborty
15	Interphase Mass Transfer and Reaction in Immobilized Enzymes	Saikat Chakraborty
16	Interphase Mass Transfer and Reaction in Immobilized Enzymes (Contd.)	Saikat Chakraborty
17	Effectiveness Factor in Immobilized	Saikat Chakraborty

And simultaneously diffusion and reaction and all these things, then I will over to microbial growth.

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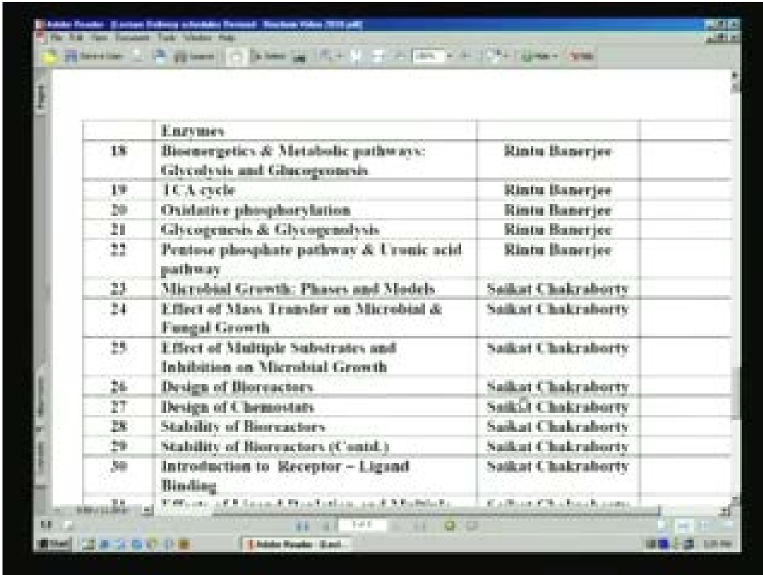

	Enzymes		
18	Bioenergetics & Metabolic pathways: Glycolysis and Gluconeogenesis	Rintu Banerjee	
19	TCA cycle	Rintu Banerjee	
20	Oxidative phosphorylation	Rintu Banerjee	
21	Glycogenesis & Glycogenolysis	Rintu Banerjee	
22	Pentose phosphate pathway & Uronic acid pathway	Rintu Banerjee	
23	Microbial Growth: Phases and Models	Saikat Chakraborty	
24	Effect of Mass Transfer on Microbial & Fungal Growth	Saikat Chakraborty	
25	Effect of Multiple Substrates and Inhibition on Microbial Growth	Saikat Chakraborty	
26	Design of Bioreactors	Saikat Chakraborty	
27	Design of Chemostats	Saikat Chakraborty	
28	Stability of Bioreactors	Saikat Chakraborty	
29	Stability of Bioreactors (Contd.)	Saikat Chakraborty	
30	Introduction to Receptor – Ligand Binding	Saikat Chakraborty	

So, essentially when you try to in the in the lab you know some of you some point of time might one to incubate cells right.

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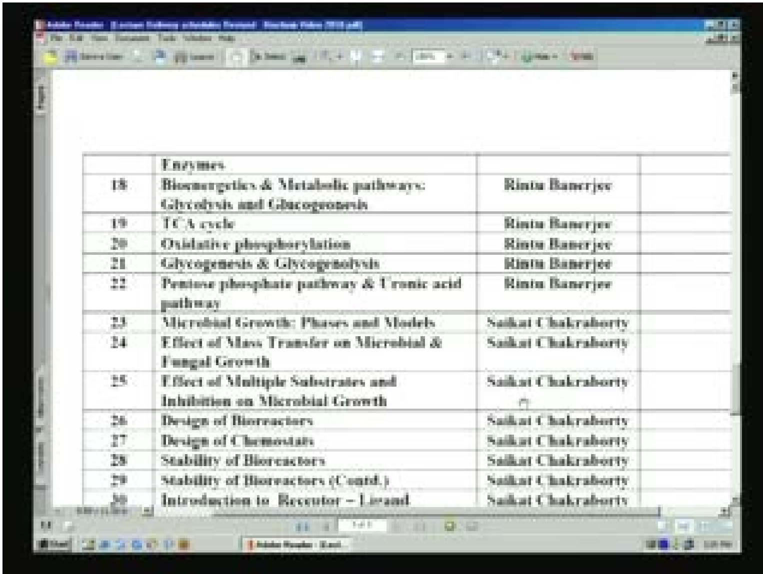

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So, what is a process of that, what is the dynamics of that incubation, what are the ingredients that are necessary? So, you start with 1 million cells for example, and by the end of one day you want to have 2 or 4 million cells of band of two days you want to have 8 million cells and so on. So, that is the way you grow cells right why do you grow cells this way, because you want to mimic in a way the growth process cell growth

process in in in living systems right and and also because you need these amounts of cells to do the experiments.

So, when you need these amounts these cells to do the experiments. So, how to do you achieve that cell growth, a. b, what are the ingredients of the cells growth c b. c, what are the kind of reactions that happen during the cell growth and d, what kind of models you can come up with both continuous stochastic to try and quantify these cell growth. So, these are aspects that we are going to look at over here.

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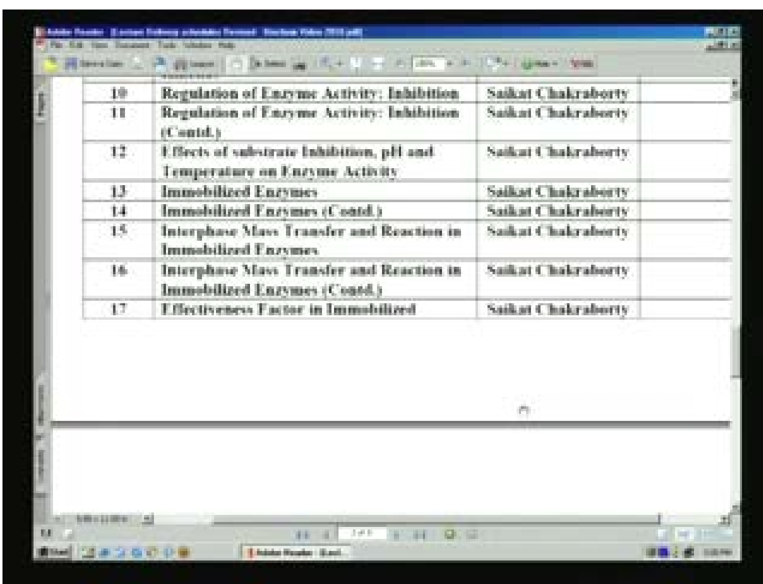

10	Regulation of Enzyme Activity: Inhibition	Saikat Chakraborty	
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Now, once we have looked at cell growth now, when these cells grow after these cells grow grow or when these cells are growing in both cases you can you can engage them in biochemical reactions in reactors.

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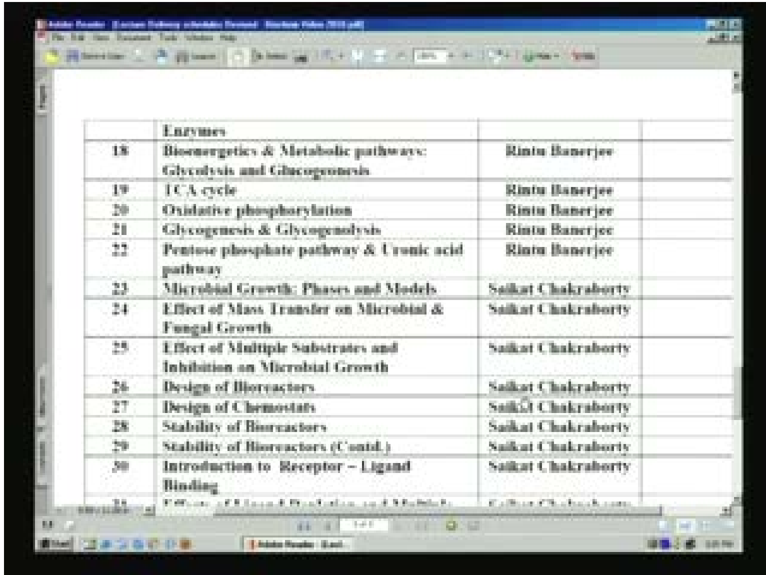

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So, these reactors you know chemostat, these are called chemostat; chemostat are nothing but, continuous c s t r continuous stirred tank reactor. So, but in the biological framework in the biochemical framework you know (( )) called chemostat. So, we will try and understand the dynamics of it, the design of it and some of the pretty interesting things we will do which you probably have not done in other courses, the stability analysis of bioreactor.

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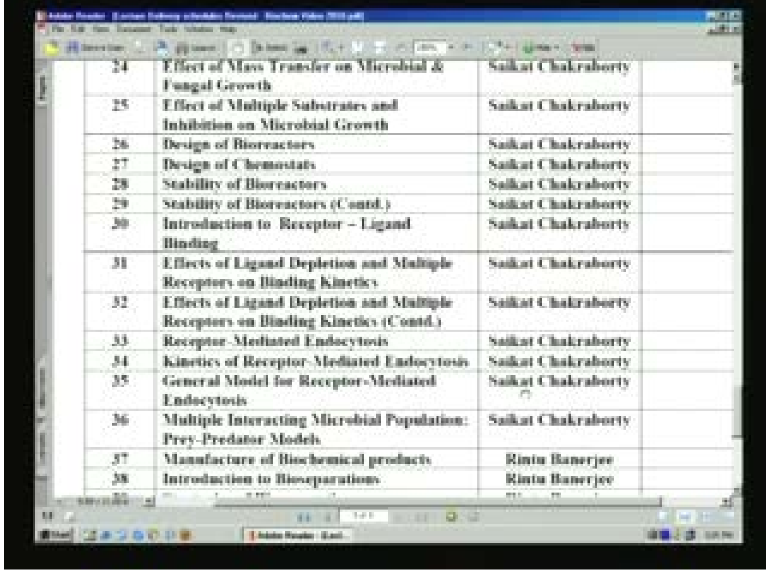

24	Effect of Mass Transfer on Microbial & Fungal Growth	Saikat Chakraborty
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29	Stability of Bioreactors (Contd.)	Saikat Chakraborty
30	Introduction to Receptor – Ligand Binding	Saikat Chakraborty
31	Effects of Ligand Depletion and Multiple Receptors on Binding Kinetics	Saikat Chakraborty
32	Effects of Ligand Depletion and Multiple Receptors on Binding Kinetics (Contd.)	Saikat Chakraborty
33	Receptor-Mediated Endocytosis	Saikat Chakraborty
34	Kinetics of Receptor-Mediated Endocytosis	Saikat Chakraborty
35	General Model for Receptor-Mediated Endocytosis	Saikat Chakraborty
36	Multiple Interacting Microbial Population: Prey-Predator Models	Saikat Chakraborty
37	Manufacture of Biochemical products	Rintu Banerjee
38	Introduction to Bioseparations	Rintu Banerjee

So, and substrate inhibition and multiple steady state and so on and then, as I said we will go on and do enzyme you know the receptor-ligand binding, which is also pretty interesting thing because, one particular disease that we are going to concentrate on you know we are going to talk about the process itself, but with the example of of one particular disease, which is I will give hint and you have to tell me the name of the disease, which is very common in India, it seems like 1 of the 50 percent of the people who suffering from disease or something like that are from India, so and it has to do with can you tell me some names first, and then I will give you some hint.

Tuberculosis

Tuberculosis is no 50 no, so many people in India suffer this, this is like common disease and this is more of the middle class disease you know people, what is that?

Diabetes

Not diabetes, but you are close what are the other diseases? Something does with lipids something do with lipids fats.

Obesity

Not obesity, but yes it is called with the name scientific name of the disease is familial hypercholesterolemia you you heard of it hypercholesterolemia, high cholesterol you have not heard of this. So, it is called familial hypercholesterolemia and why is it called familial hypercholesterolemia? Because, the familial part is has to because its family thing. So, we will try and understand the effect you know the effect of gene expression and so on and this. So, it is familial, so it it transfer from its genetic disease its transfer from one generation to another.

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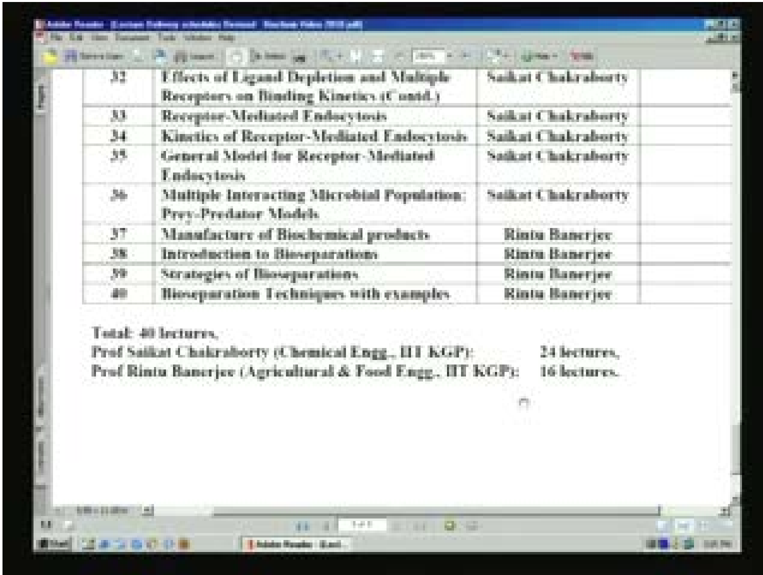

32	Effects of Ligand Depletion and Multiple Receptors on Binding Kinetics (Contd.)	Saikat Chakraborty	
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37	Manufacture of Biochemical products	Rintu Banerjee	
38	Introduction to Bioseparations	Rintu Banerjee	
39	Strategies of Bioseparations	Rintu Banerjee	
40	Bioseparation Techniques with examples	Rintu Banerjee	

Total: 40 lectures,
Prof Saikat Chakraborty (Chemical Engg., IIT KGP): 24 lectures,
Prof Rintu Banerjee (Agricultural & Food Engg., IIT KGP): 16 lectures.

And hypercholesterolemia hyper means extra and cholesterolemia, extra cholesterol. So, anyway I think what we will do now is let us move on to this is just wanted to give you a sense of the whole thing and let us move on to the lectures.

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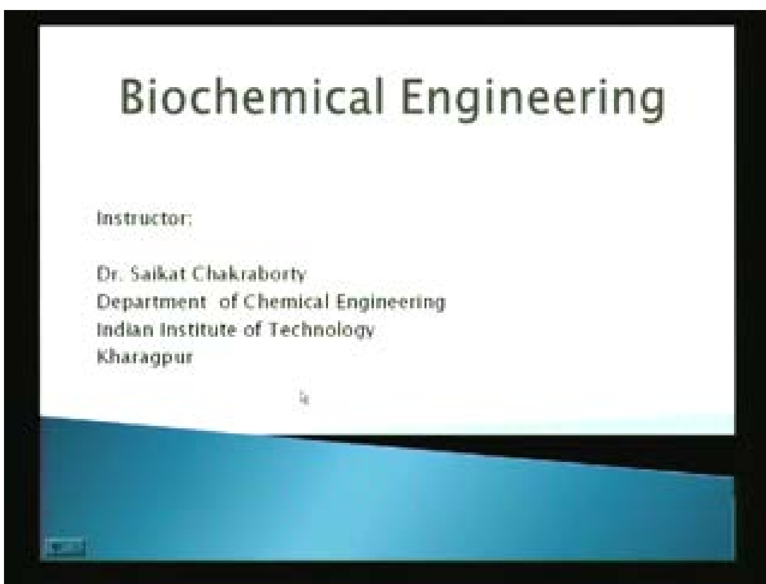

So, you know, so as I said that enzymes essentially are you know proteins RNA or DNA that catalyze biological reaction.

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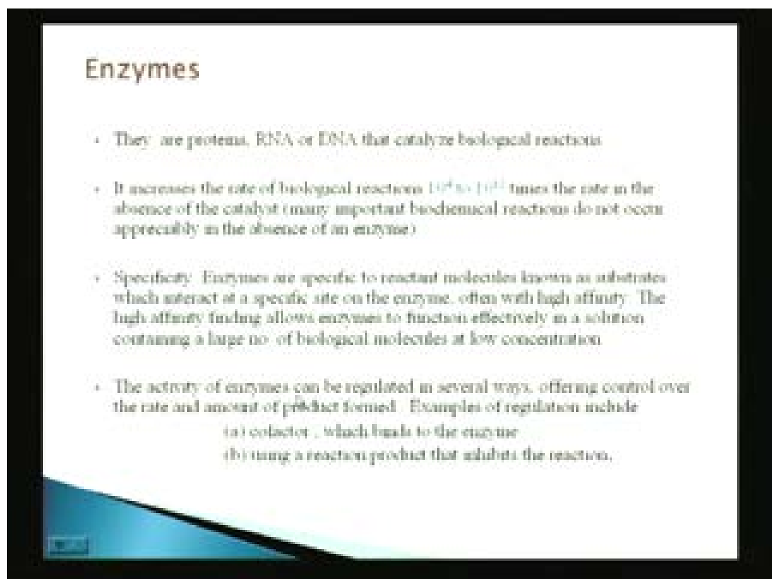

So, essentially function of enzyme is to catalyze the biological reaction and there is a huge range of enzymes that work and sync with each other in the human body and without these enzymes, the human body as you know it would not function.

So, they form a very integral part of of of the system and some of you are working on biological system you know this. It turns out these enzymes catalyze the rates of biological reactions by 10 to the 4 to 10 to the 12 times, if there is no catalyst and in the if there is catalyst, so the comparison between them. So, when you put enzyme in there it catalysis 10 to 10 to the 4 or 10 to the 12 times the rate that in the absence of catalyst.

And and as as I said here you know one of the points are noted here is that most important biological reactions, do not occur in the absence of enzymes because, these enzymes even if and trace quantities of them really works. So, you do not need a lot of them actually. A very important thing about thing about enzymes especially in the human system is there is specificity, what I mean by specificity is that, these enzymes are specific to reactant molecules. So, you want one set of substrates only or not one may be two sets of substrates only to react with that enzymes and that is way they called specific.

So, unless these enzymes are specific, there is no point to including them, because see you might have host and 1000 and millions of reactions working in the occurring in the human body. So, you need one set of enzymes to work for one set of reaction, otherwise there has been incomplete kiosk and how this that is ensure is that, the substrates have

high interaction in specific interaction affinity, high affinity with that particular enzymes and I will show in a minute how that you know how how that really happens? The high affinity finding allows enzymes to function effectively in the solution containing a large number of biological molecules at low concentration. So, because it is very specific, so even if you put a little bit of enzyme in in a large amount of solution, though actual concentration of the enzyme is very low, but as a result of the fact that, it is very specific it works. So, specificity is the most important thing in in enzyme activity.

And then, there is something like regulation of enzyme activity. So, you know you may not want a lot of enzyme working or you know working at the same time. So, for example, in catalyst activity also some of few you worked with catalyst you know that you need to control the catalyst activity right. See, you do not uninhibited catalyst activity. So, just enzyme is catalyst. So, there are ways to control the enzyme activity and the body finds its way and what we might what will study in this part of this course is, how these enzyme activities are regulated. So, that is called regulation of enzyme activity.

So, what we will studies how these enzyme activities regulated by the human body and how we can mimic that and there are ways to regulate these enzyme activities externally also, because once we understand what the process of regulation is and once we quantify, there are way to give in a better to regulate these enzyme activities you see what I am saying. So, the body itself does it internally, but then once you understand the mechanism of this regulation, you can do that externally as well right. So, that is another thing and so we use cofactors and so on and we are going to study that.

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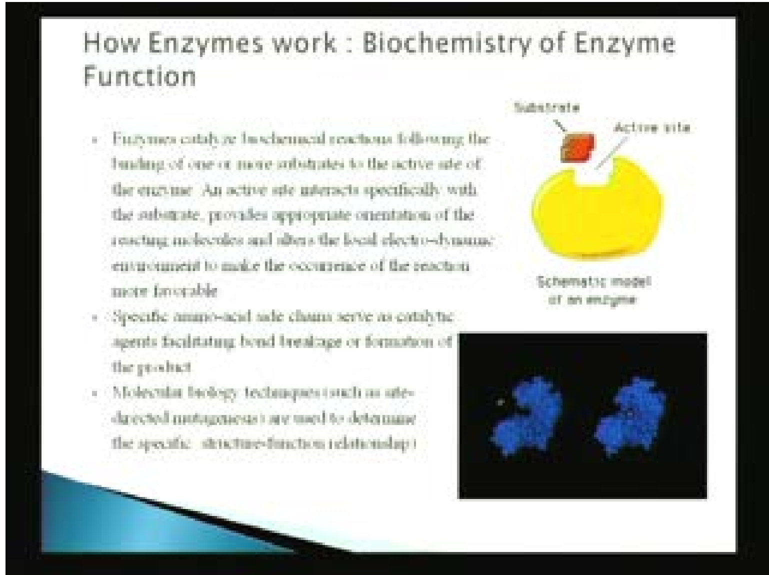

So, now let us come to the question of the biochemistry of enzyme function and this is essentially what we want to study in this lecture. So, how do these enzymes work and after we have done biochemistry, we will move on to the thermodynamics of it, why I want to do is let me show you some pretty pictures.

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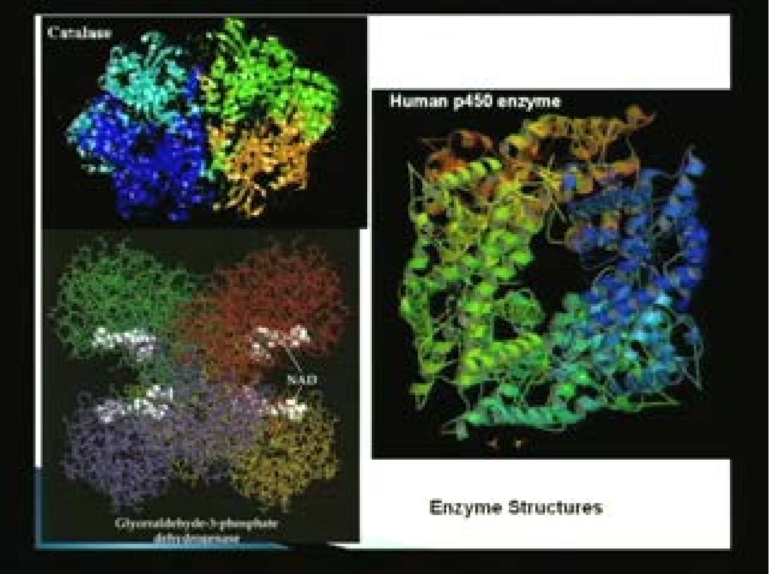

So, these are pictures of some of the enzymes you know few enzymes. So, this is what you see on left top corner this is catalase what you see here is the glyceraldehydes 3 phosphate dehydrogenase and other enzymes this is the human p450 enzyme, what you

see essentially these bunch of RNA's and DNA's and proteins cling together and forming these ribbon like structures and so if you go back now, what happens now I hope you saw this. So, I am going back to the older slide.

So, now to the understanding of what really happens. So, enzymes catalyze biochemical reactions following binding. So, essentially what happens is the enzyme is there and there is something called an active site look at this picture here. So, this is the enzyme yellow thing is is schematic of an enzyme what you have is called an active site part of the enzyme, which is known active site just like an absorption you study this in the absolution also right, there are active site for absorption.

So, what happens the substrates comes and bind to the active site, it is very simple thing, but then there are other complications that come in at some point of time. So, once it binds, once the substrate binds then, it starts to function. Now, if you look at the geometry of it, can you make some comment on what why this geometry is like this of the active site and the substrate?

(( ))

Specificity very good do you get what he said. So, this is like lock and key mechanism. So, look at the look at the structures of the substrate and the structure of the active site and the kind of fitting to each other very appropriately. So, that is the one that that the stereochemistry is one that ensure the specificity of the enzyme. Now, is there a way so I think, if you if you do this stereochemistry, if you understand the stereochemistry of the process, is there a way to say enzyme is made for substrate one, is there a way that I can allow substrate to to react with the enzyme, what is that?

(( ))

Absolutely to mimic the stereochemistry, so I design my substrate to to mimic the stereochemistry of that and I think one of the examples I gave you once was that, the morphine and the heroin thing that, that is what is you know this is blood blood brain barrier is there. So, which is which cannot be otherwise overcome, unless it couples with some substrates some enzymes? So, what is done, what doctors do is they try to come medico, they are trying to come up with these morphine's, which have the same stereochemistry of the heroin and that can cross the blood brain barrier.

So, you allow morphine also to cross blood brain barrier by mimicking the stereochemistry of that. So, this is one of the thing we do we can do over here. So, you have the substrate one and if you want substrate two out there to bind to that and you mimic the stereochemistry of substrate one make it look like it is like coming up with (( )) key to the lock and you want to enter (( )).

So, an active site interacts specifically with the substrate providing appropriate orientation. So, this has to do with stereochemistry of the reacting molecules and is also the electrodynamic environment to make the occurrence of the reaction favorable. So, I will I will come to that, when you come to the thermodynamics part you will understand this little better that, how it alters the electrodynamic environment to make the reaction to facilitate the reaction.

And in terms of biochemistry, there is specific amino acid side chains that serve as catalytic agents facilitating bond breakage and formation of the product and what we do is there are several molecular biology techniques such as mutagenesis and so on, that are used to determine the specific structure function relationship and this is pretty important the reason is that, see there is a direct relationship between the stereochemistry of the function structure of the enzyme and the function is performance right and how an enzyme facilitates the particular reaction is also govern the influence by it structure.

So, and that is way it is very important to understand the structure function relationship and if you want to you know one of the things that are important in the human human drug preparation and delivery is to come up with some important important enzymes, which can govern or which can influence biological physiological processes. So, unless we understand the structure function relationship of existing enzymes, so that is what I trying to say that, if you want to mimic the activity of the of the function of a particular enzyme that is already existing and come up with something new and innovative you have to understand the structure function relationship where in once relationship that is there between structure and function of that enzyme in order to be able to mimic that right.

And once of you know modern biology and you know it some people said that, recently that its well known recently that this century is going to be the probably the century of biology and if you want to do that, you have to understand the human system of the of

the system that is that is there in nature very very well what I mean by that is that, you know what we have to do is do reverse engineering, which is understand the engineering of the human body or the physiological system in itself and then, try to mimic that, why is that because, you know human system has gone through 460 million years of evolution you know. So, starting from when I will I started itself, you cannot have anything better than that in one lifetime in 100 years, 50 years, 100 years of industrial age we cannot better that.

So, the best thing we can do is try and understand the human the way physiological system or human system works and then mimic it. So, that is the reason we need to understand this structure function relationship that is not something we actually essentially going to do in this course, but I want to stress the effect importance of it to you. So, that if at any time point of you are interested you can go and you know study that. So, some of these you know for example, why these structures are like this, where the active sites and what essentially happens, when you go into the biochemistry, these are things that beyond purview, this course this is more an engineering course. But, at some point of time it is good enough to (( )) your interest in it. So, you might want to take some other course and this and there is some chemistry department.

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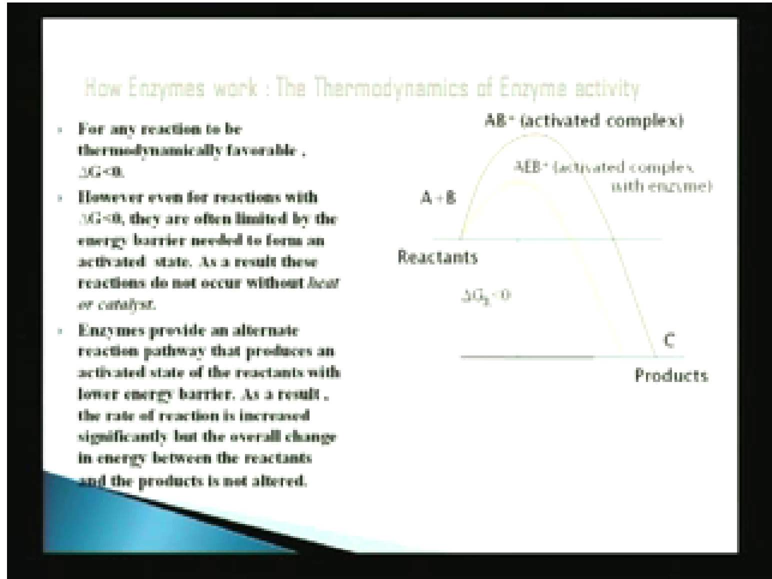

Now, let us in the next next part of this let us talk about the thermodynamics of enzymes. So, you know I would would like to you participate on this little bit, but anyway so it is

out there in front of you and not too many questions I can ask once I shown you the slide. So, say essentially you know this right that if for reaction to be thermodynamically possible your its its delta g is Gibbs free energy change between the initial and the final change, final stage has to be negative right without that we cannot have reaction.

Now, if that is not possible then, there is no role of enzyme let us be very clear about that enzymes cannot change the Gibbs free energy delta g for the system, the delta g for the system the Gibbs free energy change for the system is between the initial stage and the final stage and the energy and the enzyme has no role in it actually, what the enzyme does is what you see here in the picture.

So, if A plus B is to go C essentially it have to form an activated complex right A B plus and the problem with this is that, there is a big threshold that is there. So, you have to go reach the mountain top and then dive in right even, if your delta g is less, but you need threshold energy to cross that energy threshold barrier, what the enzyme does and we will show that you know maybe it is not in today's class we can show little bit, but very soon I mean in in the following classes we will do that, what the enzyme does essentially create a different complex.

So, A and B together form a complex A B plus and activated complex A B plus which has a certain which has a certain you know activation activation energy. So, this is this is your activation energy what the enzyme manages to do, it binds to the active sites of the substrate of substrate rather binds with binds with to the active site of the enzyme, it forms a different complex which in this case is written as AEB plus E being the enzyme and this complex has much lower threshold energy of barrier to be to be jumped across to go to C.

So, that is essentially is is the thermodynamics of enzyme. So, it turns out that; however, even for reaction with small delta g, there often limited by the energy barrier and you cannot perform these reactions without catalyst, there are two kinds of things that I mentioned this in the first slide itself that, two kinds of possibility; one is the reaction occurs without the enzyme and you use the reaction to use the enzyme to catalyze it, to (( )) you know accelerate it, there is another possibility, where the reaction does not occur at all with the enzyme, why is that, thermodynamically it might look ok, but the point is that, so the delta g could still be negative for that reaction.

So, the reaction delta g what is delta g negative tell you delta g negative does not tell you the reaction going to occur what it tells you that the possibility of the reaction to occur, that it is possible for the reaction to occur, if you look carefully and go back to your thermodynamics book that what you will find it does not say that reaction is actually going to occur. So, it just ensures the possibility that it this feasible to conduct this reaction, now there are plenty of reactions, where the delta g could be negative, but the threshold the initial threshold from from the initial state to the complex is so high, that the reaction does not occur unless, you put an enzyme. So, the that is the very important thing and many biochemical reactions are like that.

So, So enzymes how to do it and as I said you know this is just summary what is written here, is a summary of that the enzymes provide an alternate reaction pathway that produce produce an activated state of the reactants with lower energy barrier. As a result the rate of reaction is increased significantly, but the overall change in the energy between the reactants and products remain the same right is it clear, is there any question on this at this point.

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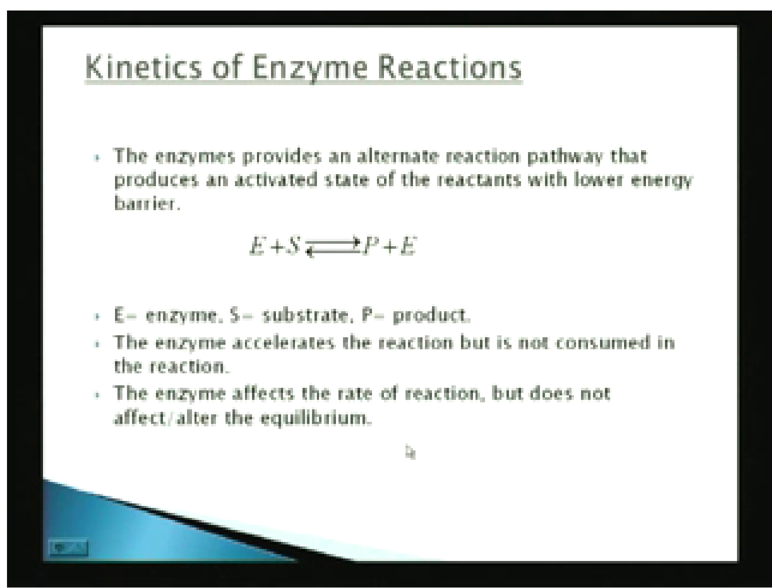

So, we will start little bit and do little bit of the kinetics of enzymes. So, essentially you know if you have reaction like S going to P, S being the substrate and P being the product right. So, without the enzyme, reaction is very straightforward we just looking at a simple reaction, the reaction is very straightforward, it is S going to P reversible by the

way let me ask you this question of this point. So, if it were not a reversible reaction, S going to P, what if you put a reversible sign there what does it mean, if I just put S going to P as reversible just one one sided arrow what does it mean?

(( ))

Just S going to P, if I put reversible sign does what does it mean, does it mean anything.

(( ))

This right

(( ))

Very high what?

(( ))

Right. So, if you put irreversible sign what does mean? It does not mean the reaction is irreversible, it only means that the forward reaction the rate of reaction is very very high as compared to backward rate of reaction. See, there are no reaction which completely completely irreversible, when we put this is something that you probably have not learned, when you put a irreversible reaction you think you tend to think that the reaction is reversible, that is not true. There are no reactions, which are completely irreversible when you put forward rate of reaction it means that, the forward rate of reaction, when you put one sided arrow it means that the forward rate of reaction is very very high as compared to compared to the backward reaction and that is why it appears in most all practical cases with or without catalyst it appears to be irreversible.

So, here when you come to, so this E is enzyme, S is substrate, excuse me P is the product and product and what happens the substrate is go back to this. So, the substrate goes and binds to the active site of the enzyme and then, you have the activated state here A. So, if you have A plus B together. So, what happens there are two substrates that that is there and the enzymes go and bind with them. So, that could be substrate here and there could be another substrate on the other side and the enzyme kind of fix into the active sites of both of them together and form this complex AEB, which then later disassociates to give you product and your enzyme.

So, the enzyme being of catalyst you know this is very straightforward, but it is important factor known to because, this may mistake sometime that enzyme being catalyst its consumed in the reaction right, it it just accelerate the reaction, but it is not consumed in the reaction and it affects the rate of reaction, but it does not have any any any affect on the equilibrium. Now, if I ask you you know for example, two two things that we do in what is the one of the major difference between say thermodynamics of of this is thermodynamics right the thermodynamics of reaction and the kinetics of reaction.

(( ))

Feasibility and its feasibility of course, but apart from feasibility what else?

(( ))

Both the both feasibility that is whether delta g is zero, less than zero or not whether the reaction is possible A and B about the equilibrium state final equilibrium state that is reach. Kinetics deals with the dynamics of the process. So, how fast that is going to be there, these concept that have to be very clear in your head for example, when you doing dealing with you know you do courses like thermodynamics and heat and mass transfer and so on.

So, what is the difference between thermodynamics when you do it the way you do and heat and mass transfer, the same difference that is in thermodynamics you talking of the equilibriums state you talking of the final state that has been attain whereas, all kinds of transfer transport processes that you do heat mass momentum transfer about the process about the dynamics of the process. So, thermodynamics time is not not variable, the variable that is discounted in thermodynamics is time, time is not variable thermodynamics. Where in other processes thermodynamics time is variable?

So, even here when we talk about kinetics, time is variable and you have to talk about the dynamics of the process and you have to find out how the rate of reaction is increased or decreased by enzymes because, see at the end of day why are we using these enzymes a to decrease the threshold that is the reaction actually occurs and b also to accelerate the reaction. So, these are two different issues and in order to understand how how it accelerates the reaction you have to look at the dynamics of the process. So, that is what we are going to do, I am not sure we can finish this today.

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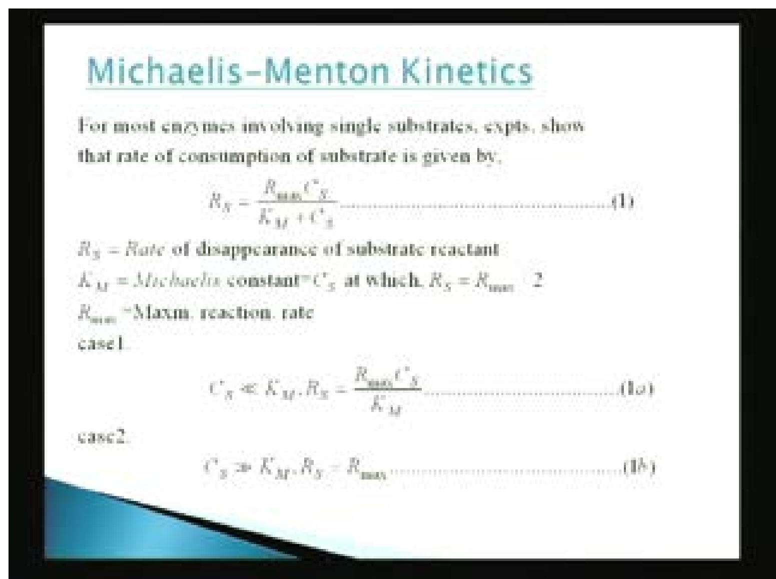

But, let me start with it at least for the next 5 to 10 minutes the classes till 9:25 right yeah. So, So the kinetics that is used and I do not want to you rush want to you rush through this, but just give you a sense of it is known as Michaelis-Menton kinetics and I will talk about this little bit these were discovered by two gentlemen Michaelis and Menton separately I think in the early part of the 20 th century early part of the 20 th century I can give you the number in the next class is in the book Bailey book also.

So, So what is important this is the most used kinetic and they they become really popular people, because this turned out to be the most used you know kinetics in in most of reactions the reason is that, if you look at this kinetics can you can you can you comment on see equation 1 that you have over here this gives rate of reaction.

So, these are for most enzymes what it says for most enzymes involving single substrate experiment shows that, the rate of consumption of the substrate is given by R s is R max C s over K M plus C s, what is C s is C s is concentration of the substrate, K M is known as Michaelis constant or Michaelis-Menton constant and K M is the valve of C s, when R s equals half of R max, R max is the maximum reaction rate that is that it is going to attain can you comment on the nature of this; if I am to plot R s versus C s that is the reaction rate versus concentration that is how you figure out what the rate order of the reaction rate right because, you have first order reaction you know it is just straight line

if you have second order is quadratic and so on. So, can you comment on the on the nature of this?

(( ))

No, but what is how what is the nature of the plot, that is written here you do not.

(( ))

Convex no

(( ))

Initially linear, so that is written there also, I am I am coming to this let us look at so whenever you are trying to understand or quantify something complicated the strategies the engineering strategies and this can you can have down to your head, the engineering strategies to look at the two asymptotes; the smallest and the largest value that that will give you what what will the asymptotes give you.

(( ))

The bonds it is called the bonds the asymptote will give the bonds of the curve. So, if you look at this for example, in here so let me go through this one more time. So, R s is the rate of disappearance of substrate of the rate of reaction, R max is the maximum rate of reaction, C s is concentration of the substrate, K M is Michaelis constant like a rate constant and C c s is substrate I told you and k the value of K M is evaluated by this. So, R s equals R max over R max over 2. So, at R s equals R max over 2, so if you put R max over 2, then the value of C s you get is that R you know equals C s at which R s equals R max whichever way.

So, you find this C s at which the rate becomes half of. So, you should performing experiment you trying to measure the the rate constant, the rate as well as the concentration. So, you measure the concentration, measure the rate at that particular concentration, how do you evaluate your K M the simple way is that, you figure out where it goes to the maximum and then half of that maximum.

(Refer Slide time: 37:42)

Derivation of Michaelis-Menton kinetics(contd.)

using equ 8 on equ 5 $\quad C_{ES} = \frac{k_1 C_S C_E}{k_{-1}+k_2} \quad (9)$

Substituting equ 9 into equ 6

$$C_E = \frac{(k_{-1}+k_2)C_{E0}}{(k_{-1}+k_2)+k_1C_S} \quad (10)$$

$$C_{ES} = \frac{k_1C_SC_E}{k_{-1}+k_2} = \frac{k_1C_SC_{E0}}{(k_{-1}+k_2)+k_1C_S} \quad (11)$$

Substituting equ 10 & 11 into equ 4

$$-R_S = \frac{dC_S}{dt} = -k_1C_SC_E + k_{-1}C_{ES}$$

$$= \frac{k_1k_2C_SC_{E0}}{(k_{-1}+k_2)+k_1C_S} = \frac{k_2C_SC_{E0}}{\frac{(k_{-1}+k_2)}{k_1}+C_S} \quad (12)$$

Let me show you some plots here.

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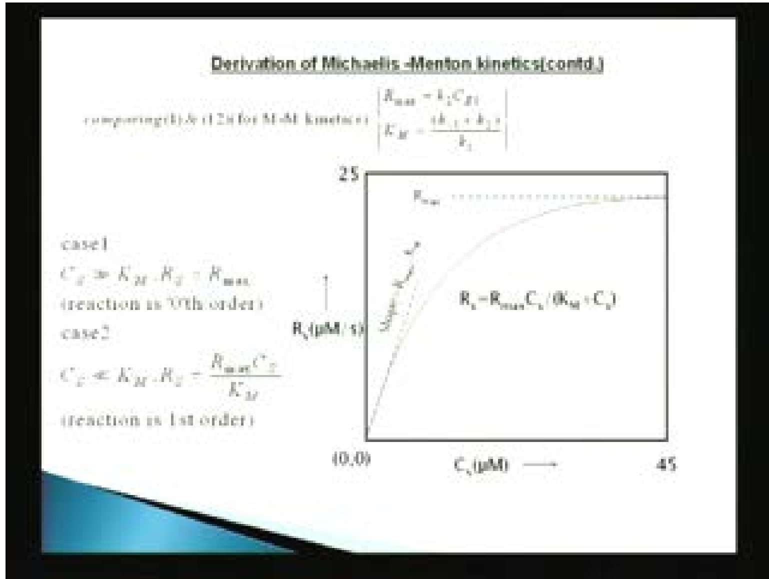

See here, so you this this is how the plot looks like and so this is R max. So, you figure out that, when this is R max say 22 or something, then at value of C s at what value of C s the reaction reaction rate is half of the maximum that is your K M. Now, so here we are so these are the two asymptotes of the of the problems this asymptote and this asymptote.

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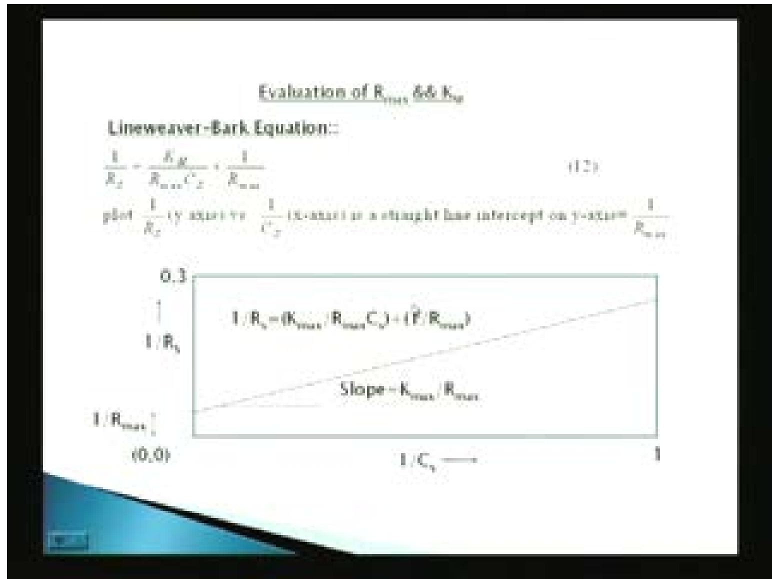

And the two asymptotes go back to the two asymptotes and try I understand that. So, for C s much much less than K M here, if you if you look at equation 1 for C s much much less than K M what happen is C s goes goes away from the denominator, but is stays in the numerator. So, R s is given as R max C s over K M fine. So, this looks like looks like a first order reaction right.

So, as a result obviously it is linear. So, if you look at the plot here look at this. So, in this small range C s very being very very small, its look like first order reaction its linear curve, what about this in the other range that is C s much much greater than K M. So, K M goes away from the denominator, C s remain C s in the numerator as well. So, you get R s equals R max fine. So, this is a zero order reaction. So, the two bonds of the whole reaction is zero order and first order which means that, what is the order of the reaction?

(( ))

Fractional order, so the order of the reaction, so it bonded by the zero and first order. So, this is the this is the this is this is how the curve looks like I need to what we will do is, we have just couple of minutes I think, because at this point I am not going to analysis today what we will do we will resume with the analysis tomorrow. So, I just give you few minutes, if you have some questions on the on the whole thing you can ask me.

So, let us let us walk through the thing. So, some of things about this let me summarize for you what we did some of things that you need to remember is that, enzymes are catalyze a, they have two roles; one is to to make reduce the threshold of the energy and make the reaction happen because, there are lots of reaction that would not happen that is why threshold the energy barrier the threshold energy barrier is so high, there are plenty of reactions in the human system that would not happen without enzymes.

So, they have two roles; one is to lower the threshold so that they reaction happens and b is to b is to accelerate the reaction rate. So, some of the numbers that I gave here are 10 to the 4 to 10 to the 12 times it accelerates the rate of the reaction on in the human system and there are plenty of reactions as I said, which do not even happen without the presence of enzymes.

Now, one of the most important things about enzymes is this specificity. So, substrates because there are so many substrates and you know you have limited number of enzymes. So, if all substrates start to react with all enzymes what will happen? The enzymes will be depleted and the reaction particular reaction that the enzymes meant for that would not be available for the reactant reactant right. So, there is a sense of specificity. So, enzymes are specific how is the specificity inactive here you are going to see.

So, the enzymes and the substrates has a sort of lock and key mechanism and if in most cases it is not that this this is simple example, where an enzyme is binding to a single substrate, but in most normal cases what that what happens, there are two substrates right and the enzymes bind in a way that you know it binds both of them or to one of them when the one is bond to the other you see what I am saying. So, either so this substrate that you see over here either, is going to bind to two of the A and B together in a way that a part of it fit fits into the active site of A and the part of it fits into the active site of B or in a way that it binds to one of them.

So, these are specific you know amino acid side chains and catalytic agents facilitating bond breakage and so on and I showed you some pictures of that and this is this is thermodynamics of it we went through that and delta g has to be less than zero for the normal reaction, for reaction to be feasible and the enzyme forms what it does it forms a

complex which is much lesser threshold than A plus B much less lesser complex and when it A plus B is formed and and and then, we went to the kinetics of it.

So, you say you know I think some of you would have to leave, because your classes so can you is there any questions let me let me address them at this point of time and then, there will be some other things I can talk about (No Audio from 43:01 to 43:24).

I think you know it would be hard to start the next section, which is the derivation of the Michaelis-Menton kinetics today. So, what we will do is we are going to start with this tomorrow in tomorrow's lecture and yeah, if there is any questions you know couple of minutes we have you can ask and otherwise (( ))(No Audio from 43:43 to 44:17). So So, what I will try to do is try and summarize one more time what we had been trying to do today which is that, enzymes are essentially catalyze biological catalyze that do two things; one is they lower the threshold energy and so that reaction can occur.

And if I go back to this slide slide as I said that, if you have reaction of A plus B going to C, if the reaction to has to go through through an activated complex A B plus and threshold energy is needed to cross the activation energy barrier and if that that complex that is formed is A plus B and product that is formed C from there, but there are many enzyme many reactions, where this this energy barrier cannot be overcome and what the enzymes allow us to do?

It allows us to form this complex A E B plus, where E enzyme A and B being the two reactants and which is the much lower activation energy barrier, which could be crossed easily, the delta g remains the same of the of the whole process, it has to be negative. So, that is one thing and the other thing is that, you know it process it accelerate the rate of the reaction. So, essentially it provides alternate pathway that produces this different activated state and as a result of that, the rate of reaction is increase significantly, but overall changes are not there in the energy and the in and both in the energy and in the products that are formed.

So, as one can understand from this very clearly, the enzymes do not take part of the reaction. So, if you look at the kinetics the basic kinetics of enzyme what happens is that? The enzyme E react with substrate to form product P and the enzyme E is again rerelease which means that, it does not take part in the reaction.

So, when you are look at the reaction overall the the enzyme E does not take part in the reaction, but in the next few lectures we will show that, how the what is the what are the steps that happened in between between this E plus S giving P P plus E there is the whole you know whole network of steps that happen and we will try and expand on that and try to show, how the enzymes actually participate in the reaction, but the net effective that, it does not participate looks like, it does not participate. So, it accelerate the reaction, but it consumed in the reaction and and it affects the rate of reaction, but does not alter the equilibrium.

The reason for that, equilibrium is guided by the thermodynamics thermodynamics and the delta g of the process, which remains the same with or without enzymes, what enzyme does it changes the dynamics of the process and it lowers the activation barrier.

So, you know so final wrap up on on today would be that, what is kinetics that it enzymes follows; in kinetics that enzyme follows follows this known as Michaelis-Menton kinetics, accept very well known kinetics and what it does the kinetics says that, the rate of reaction so when this reaction between of E plus S giving P is there what is the rate at which S being produced, that is rate dependence of course, on C s, but if it were in the absence of of of of an enzyme for example, it would be a first order in s or it could even be a second order in s, but the way this reaction is written over here it would have been first order reaction in s right.

But, as it turns out that when you have the enzyme the the kinetic of the processes is altered and what you have is known as Michaelis-Menton kinetics, it is not a first order or the second order or zero order or any kind of that and the expression of the Michaelis-Menton kinetics and I will do summary of that you know go back to it again tomorrow in tomorrow's lecture, what happens is that, this rate of reaction R s is given as R max C s over K M plus C s, R max being the maximum rate of reaction, C s being the concentration of the substrate, K M being the Michaelis constant.

The reason I mean this this is so popular and so important is that, it turns out many and many of the biological processes that are catalyze by enzyme follow this reaction kinetic and K M is known as Michaelis constant, which is the way its evaluated is that we measure keep measuring so this is the plot for example, of R s versus C s and keep measuring the values of the rate, rate rate at different values of concentration and when

the value of the rate reaches the maximum, it saturates out. So, this is how the graphs look like.

So, at very low values of C s, the plot has a linear nature and if I go back to this at very small value of C s, if I put this back into equation 1, what I find is that so C s I can neglect in the denominator whether C s stays in the numerator and if I go back if I use some equation, equation 1 what I find is R s turns out to be R max C s over K M and this is the first order reaction, because R s varies directly with C s.

Now, at very large values of K M, C s is much much larger than K M at very small values of K M sorry C s is not much much larger than K M as a result of which K M is goes away from the denominator, C s stays in the denominator and R s is R max C s over C s. So, C s, C s cancels out and we have R s equals R max. So, this turns to be zero order reaction, now when this is zero order reaction what this means is what does it zero order reaction mean? It means that, the concentration is independent of the reaction rate is the independent of the concentration.

So, as a result of that R max is just a straight line parallel to the x axis. So, these two give me the two bonds for the reaction order. So, at small concentration of C s, the reaction order is first and it is linear. At very large concentration of reaction order is zeroth and it is parallel to the x axis. So, for all concentration in between these two, the reaction order is going to be in between the zeroth and the first order. So, the Michaelis-Menton essentially says, if you are to evaluate the order for reaction; the michaelis-menten turns out be, an out reaction of order between zero and one. So, as compared to if the enzyme was reacting directly, it would be first order reaction.

So, I think will stop here today at this point and we can continue with the kinetics of the process more detail analysis of the kinetics of the process, which is the analysis of the Michaelis-Menton kinetics and I will kind of you know come back to with the Michaelis-Menton to start with. So, because this is a very important thing and we need to understand this clearly the the way the kinetic looks and what its role before I go and analyze this little further. So, we will do this in the next class. So, I think I will stop here.

So, what we have essentially studied, we have understood what enzymes are, we have to defined enzymes understood their function and specificity, we have understood the biochemistry little bit and how the substrates you know go and bind the active site of the

enzyme, we have looked at structures of enzymes few enzymes human p450 enzyme on your right the catalase and the glyceraldehydes 3 phosphate, dehydrogenase enzyme is there also and those things.

And then, we have looked at the thermodynamics of enzyme and how it works and looked at the basic kinetics and Michaelis-Menton kinetics of enzymes and how what is the relationship between the rate and the concentration. So, we will stop here and we will continue with the details of the Michaelis-Menton kinetics in the next class, thank you.