Aspects of Biochemical Engineering
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Lecture - 21
Kinetics of Enzyme Catalyzed Reactions Using Free Enzymes – I

Welcome back to my course aspects of biochemical engineering and last couple of lecture I tried to concentrate on 2 different aspects one is that chemical reaction kinetics and another is the reactor analysis and since that biochemical engineering and chemical engineering they are related. So, first we should know the ABC of this chemical engineering in particularly reaction kinetics and the reactant analysis. Now with this information now we will enter into the real topic and today the topic that I am going to discuss that is kinetics of enzyme catalyzed reaction using free enzymes.

Now, as you know that in the microbial system if you comprises of multi enzyme system different enzyme they have metabolic pathways in the metal metallic pathways your substrate it degraded to different products now if you look at the metabolic pathways they the different steps individual steps they required individual enzymes. So, basically we considered the microbial system as a multi enzyme system. So, understand the microbial system better first we should know what is enzymatic reaction kinetics. So, this present lecture and couple of lecture I shall concentrate with this try to understand what do you mean by enzymatic reaction kinetics and first we shall have to know that that what is called enzymes.

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Enzymes: Definition

- ✓ Enzymes are **globular proteins** that act as **biological catalysts.**
- ✓ **Increase the rate** of biochemical reaction **without** themselves being **chemically altered**
- ✓ **The nomenclature of enzyme:** Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase".

Enzymes → Endoenzyme → Enzymes that function **within cells**, e.g. metabolic enzymes like cytochrome oxidase

Enzymes → Exoenzyme → Enzymes that catalyse reactions **outside cell**, e.g. digestive enzymes like amylase, lipase, protease

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Now, if you look at the enzymes is the globular protein that acts as the biological catalyst, now what do you mean by globular protein if you look at the structure of the protein it is of 2 types.

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One is globular protein and there is fibrous protein, now what is globular protein it is randomly folded you know this is like this it is randomly folded and when it folded randomly some active site might be developed, maybe this active site is developed and this configuration of this active site is similar to the structure of a particular substrate

molecule this is the enzyme and this is this. So, you know then this substrate seat here at the active site and react to give the product.

So, but in a fibrous protein is not a fibrous protein this is kind of structure like this. So, they have now this is this folded structure of the globular protein and this fibrous structure. This is mostly due to the hydrogen bond and this hydrogen bond in between the R group we know that 20 different amino acids are there are group of 20 different amino acids in combination with amino and carboxylic acid Gibbs they make a folded structure, this is how they form the active site.

Now, that the role of a rob the enzyme is a catalyst and as you know the catalyst role is they take part in the reaction and they reduce the activation energy of the reaction. So, that rate of reaction is increases and after the reaction is over it remain unaltered. So, and one specific feature of the enzymatic reaction is the enzymes are very specific with respect to substrate another thing I forgot to mention that as I told you that enzymes are globular protein, but not necessarily enzymes are protein it may be some pure organic molecules and due to advancement of organic chemistry now it is possible to produce some kind of synthetic enzyme now I can give the example of r n s.

So, another very important thing is that what do you call the nomenclature of the enzyme nomenclature it means how we name the enzymes most of the enzymes usually name ended by "ase" as for example, lipase amylase, protease like this and, but you know there is some exceptional case where some enzymes name is pepsin as you know pepsin is remaining in our stomach this is rennin it is largely used in the cheese making industry and trypsin and that is represented in our intense time.

So, that we have the usually go into the name of the enzyme is ended by ase now enzymes may be of 2 types one is called Endoenzymes and that is Exoenzymes, the endoenzymes means they walk within the cells as for example, if you look at the embden meyerhof pathway we have so many enzymes participating in the reaction and most of the reactions are there endoenzymes and all and, but the exoenzymes is basically to the hydrolytic enzyme the organism this produces and it comes out of the cells and then interact with the with the substrate and substrate will undergo the hydrolysis and give the product.

So, this is the examples I can give you the enzyme that function within the cells the metabolic enzyme like the cytochrome oxidase then we can I can give another example of phosphofructokinase of the Embden Meyerhof pathway enzymes catalyze reaction outside the cells we have different hydrolytic enzymes like amylase, lipase, and protease.

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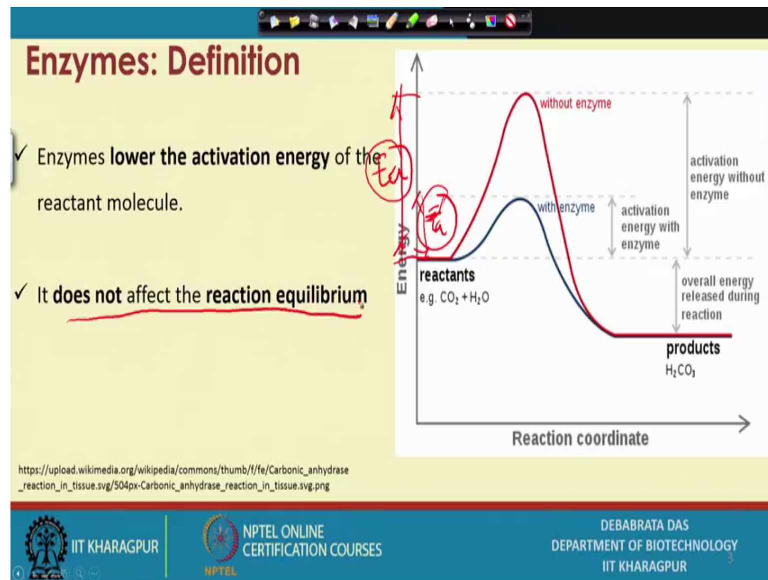

Now, I was taking a telling about that how enzyme participate in the reaction now enzymes it will lower the activation energy if you look at this figure that you know normal enzymatically reaction you require this much of activation energy this is the normal activation energy that is required.

Now, when the presence of catalyst the enzyme is there the activation energy is requirement. So, this is much less as compared to here. So, since this activation energy requirement is very less. So if you enzymes present in the reaction mixture is the accelerated the rate of reaction the pro the only the thing is that after the reaction is over the enzyme remains unaltered, but one thing I want to highlight here it does not affect the reaction equilibrium.

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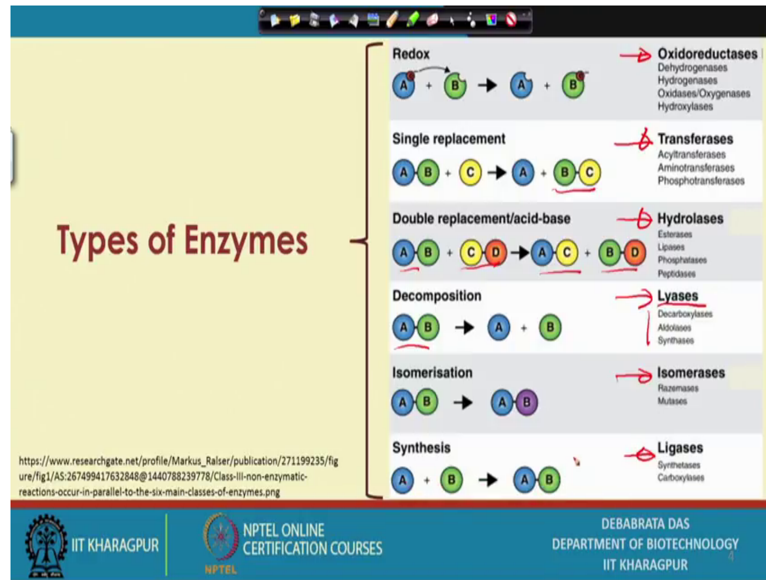

Now whatever enzymes we have it broadly it can be classified into 6 different types as for example, we have Oxidoreductases this is one enzyme, then Transferases it is another name, then Hydrolases that is another enzyme, then Lyases, then Isomerases, then Ligase. All the enzymes more or less they fall under this category. Now oxidoreductase that the enzymes who is take part in the oxidation reduction reaction what do you mean by oxidation reaction you take out 1 electron and reduction reaction will add in the electron to the molecules. The single replacement of this you can see that replay that is this is replacement that how it is taking place here this is transferases, hydrolases will hide this is water molecule this is the substrate and we need hydrolases it reproduced to compound I can give the example of sugar when sugar hydrolase in presence of water it produce glucose and fructose.

Now, another enzyme is called lyase this under go to a decomposition the exam I have given lot of examples here that decarboxylase hydrolase this is how the reaction take, isomerases is very interesting enzyme we change the functional group as for example, glucose is converted to fructose and glucose has the aldehyde group and fructose has ketone group. So, naturally that for this change transformation we require to one enzyme what they call glucose isomerase the enzyme now. So, this is the called isomerase is this then we have ligases, ligases means 2 molecules they attach together you can see this is the how enzyme classification can be done.

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Now, let me tell you that what are the different components present in the normal enzymes, enzymes has 2 components, one is called Apoenzymes and another is the Cofactors. Now what do you mean by Apoenzyme, Apoenzymes is the kind of we consider kind of it is the protein molecules, but this is inactive prom, but the Apoenzymes I told you this is inactive enzyme this is not active and. So, it will not a it react with the substrate and give the product.

But in presence of cofactor now cofactor may be of 2 types one is organ inorganic component when we have manganese ion or magnesium iron or you have zinc ion and coenzymes this is non protein molly organic molecule like NADH, FADH, ATP those molecules we considered as a. So, coenzyme basically this is the organic molecule and some other than organic molecule we consider also the cofactor inorganic molecule, now Apoenzyme plus cofactor or coenzymes we call holoenzyme, holoenzyme means active enzyme.

Now, I can this is pictorially can be represented like this now if you look at the configuration of the substrate this does not have any reason balance much of reason balance with the configuration of the active side the as soon as that your coenzyme sits here then the way you can find it is the reason balance with the substrate can sit at the active site and give the product this is how Apoenzyme is activated.

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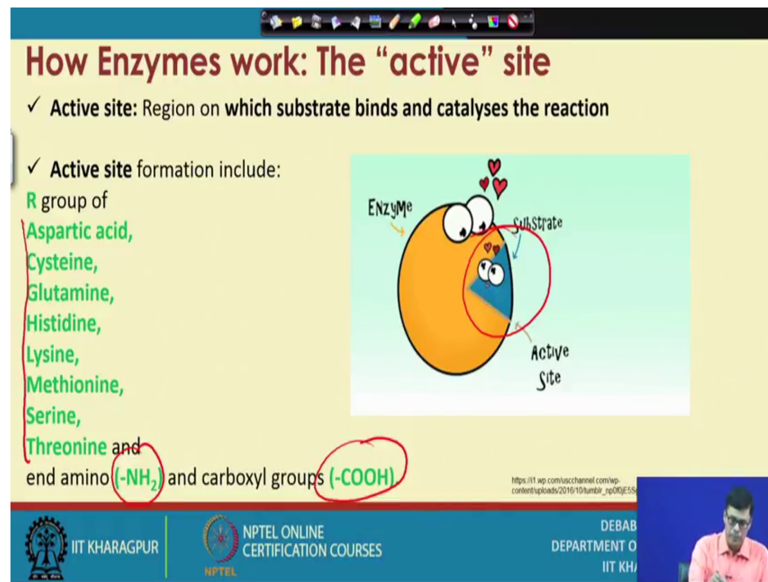

Now question comes how this active site formation take place this is very interesting as you know that we have 20 different amino acids out of 20 different amino acids of R group of 8 different amino acids like aspartic acid Cysteine, Glutamine, Histidine, then Lysine, Methionine, Serine and Threonine and this in combination with the amino and carboxylic acid group they form the this active site.

They form this active site then substrates sit here this is, so here I want to highlight only one thing that protein with the active site we call it enzyme and the protein which does not have active site we cannot call it enzyme because enzymes should you should have should react with the substrate and give the product .

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Mechanisms of Enzyme catalysis

✓ The Lock and Key mechanism: The enzymes act as a lock into which the substrate (key) fits.

Substrate= "Key" Enzyme= "Lock" Active Site= "Key hole"

Enzyme and Substrate

Enzyme-Substrate Complex

New products formed

Enzyme completes reaction; products are released

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Now, question come how substrate and an enzyme they interact with each other there are 3 different hypothesis that we have that can be described that you know how substrate and enzyme they can interact with each other.

First hypothesis the lock and key hypothesis what is lock and key hypothesis as you know that when you open a lock the key should be prepared the liberal we have in the lock we have liver system and if your key does not fit in the lock then you cannot open it. So, similar to the lock and key hypothesis means the active site of the enzyme should be the detour this is the same as the configuration of the substrate. So, they can see it at the active site if you sit properly at the active site then you will give the product, now I can give a example here you can see that it is pictorially demonstrated here that suppose this is the enzyme and this is the substrate and this has the reason balanced with the configuration of the substrate as the reason balance with the configuration of the active sites, substrate can sit here and give the product.

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Catalytic mechanisms

✓ The proximity effect : As the substrate approaches to the active site of the enzyme, the configuration of the active site changes due to **induction** so that reaction takes place.

Substrates
Product
Enzymes bring substrates to close proximity
Enzyme
Enzyme-substrate complex
Enzyme

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Now other two may the hypothesis and one is called proximity effect what do you mean by proximity effect proximity effect means when substrate approach to the active site because if apparently when substrate you look at the configuration of the substrate and configuration of the active site they are not reason balanced with each other, but as soon as the substrate approach to the active site it due to induction there will be change in the configuration of the active site.

So, that your substrate can see that the active site properly and give the product, I can give a very simple example here you look at here that this is the substrate molecule let us assume that and this is the active site of the enzyme. So, we can easily find out the configuration of the substrate and configuration of the active site they are not same, but as soon as it approaches here the configuration changes. So, the substrate molecule can sit properly and give the product.

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Catalytic mechanisms

✓ The orientation effect : Enzymes can hold the substrates at certain positions and angles due to induction to improve the reaction rate.

Now another is the orientation effect, what do you mean by orientation effect, orientation effect means suppose that when substrate. So, you know that enzyme I told you that enzyme with active sites we call it protein with the active site we call it enzyme.

Now, suppose how the protein formation take place the due to polymer of amino acids and we I told you when here I previously that if the number of amino acid in between 20 to 50 then we call it peptide and more than 50 amino acid recall it protein, the protein a now that is the polymer of amino acids now what is happening that a tiny part in a small part of the protein molecule that is the active sites.

So, suppose the active site it is your substrate is this side and active site is the other side. So, as soon as your substrate approaches to the enzyme then there or some orientation take place of the enzyme molecules. So, that substrate can sit properly at the active sites of the enzyme. So, this is the orientation effect and this is the also taking place due to induction.

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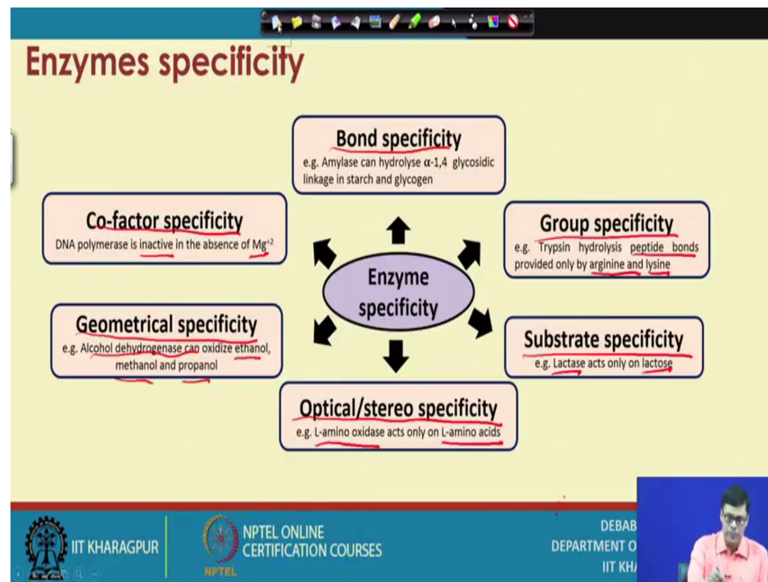

Now, I told you that enzymes are very specific as per activities concerned. So, there are you know this specificity we can divide it into 6 different categories one is called Bond specificity, another is Group specificity, Substrate specificity, Optical stereo specificity, Geometrical specificity and the Cofactor specificity I have given different examples here as for example, amylase can hydrolase alpha 1, 4 glucose glycosidic linkage because you might be knowing that alpha amylase they act on the starch molecule.

What is starch is the polymer of glucose which are bind together with the help of alpha 1 4 linkage, now group specificity that trypsin hydrolyze the peptide bond provided by the arginine and lysine, then substrate specificity which is lactase acts on lactose I have given the example of another substrate specificity like glucose isomerase act on glucose it can act on lactose fructose the optical or stereo a specificity mean the l amino oxidase act only on a l amino acids. So, we know that 2 type of optical isomers this is the L and d. So, L amino oxidase can act on L amino acid what is geometrical specificity that the alcohol, dehydrogenase can oxidize ethanol, methanol and propanol. Cofactor specificity means DNA polymerase is inactivate in presence of magnesium ion.

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Enzymatic activity

- 1 I.U. = µmole of substrate degraded per mL enzyme solution per min
- 1 U = µg or mg of substrate degraded per mL enzyme solution per min
- Specific activity: µmole of substrate degraded per mg protein per min
- Turnover number: mole of substrate degraded per active site of the enzyme per min, $K_{cat} = V_{max} / E_0$

Next point that is very important what do you call enzymatic activities the how good and bad is the enzyme that can be express with the help of exhibit now if activity can be expressed in different ways one is called I U, I U means International Unit, what is I U, the micromoles of substrate degraded or micro moles of product form per milliliter of enzyme solution per minute this is decided by the I U pack that is the international body they decided the definition of I U now if I express the enzymatic activity is 5 I U, 2 I U, 3 I U then we do not have to explain what do you mean by I U because this I U has been defined by the I U pack.

But if you express in the unit that is not I U then you have to define this maybe in different way as I have given one examples that either micro moles or milli micro gram or milligram of substrate degraded per milliliter of enzyme per minute you can experience whatever things you like, now another activity we that is called specific activity what is specific activity micro moles of substrate degraded per milligram of protein part per minute.

Now here I want to stress one thing when you go for the specific activity that implies that you know that characteristics of the enzyme that; that means, you know that have been enzymes are good or bad actually that we can easily find out with the help of specific activity because if you I U that it may vary because if you dilute the enzyme solution

then I U may vary but, specific activity will remain constant this determine the characteristics of the enzyme.

Another is very important thing is the turnover number, turnover number when the protein molecules is very big it might be there is a possibility it might be having more than one active site, in that case the activity is expressed moles of substrate degraded per active site of the enzyme per minute and K cat equal to V max by E 0 this how we can express that.

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Enzymatic reaction Kinetics

Michaelis-Menten equation for the enzymatic reaction:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

✓ Assumptions:

- When S<<Km; $v \sim V_{max}\ S]/K_m$, it follows 1st order kinetics
- When S>>Km; $v \sim V_{max}$, it follows zero order kinetics
- V_{max} α $[E_0]$

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Now, Michaelis - Menten that proposed one equation, this equation is V equal to V max S, K m plus S that this equation is purely on the basis of the correlation it is shown here and this kind of if the correlation is this and this Michaelis Menten equation is not valid. Now what does it tell, it tells at low substrate concentration when you have here then V is tends to V max, how V tends to V max at low substrate concentration then what is happening that if you this is low then I can ignore as compared to this, then this is constant the V will be equal to constant into S and this is first order reaction, it follows the first order kinetics.

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Enzymatic reaction Kinetics

Michaelis-Menten equation for the enzymatic reaction:

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

✓ Assumptions:

- When S<<Km; $v \sim V_{max}\, S)/K_m$, it follows 1st order kinetics
- When S>>Km; $v \sim V_{max}$, it follows zero order kinetics
- V_{max} α $[E_o]$

Now in case we S is very high then we can ignore that then S will cancel then I can write V tends to V max. So, this is exactly happen here the where we this is then what is the reaction followed a 0 order kinetics because V will be equal to K into S to the power 0 the 0 order kinetics and maximum velocity of reaction is deep it is proportional to the then initial enzyme concentration.

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Enzymatic reaction Kinetics

A simple reaction scheme for the saturation kinetics involves two steps:

- A **reversible step** for enzyme-substrate formation

$$[E] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [ES]$$

- An **irreversible disassociation step** for enzyme-substrate complex

$$[ES] \overset{k_2}{\rightarrow} [E] + [P]$$

The **overall scheme** can be given as: $[E] + [S] \overset{k_1}{\rightarrow} [ES] \overset{k_2}{\rightarrow} [E] + [P]$ (1)

Now, Michaelis Menten proposed this equation purely on the basis of correlation between velocity of reaction and substrate concentration later on the Bricks and Hellen they proposed the enzymatic reaction kinetics to justify Michaelis Menten equation what they told the enzyme and substrate they reversibly react and from E S and when E S

formed then it produce enzyme and product now what do you mean by that suppose I told you that when you are substrate approaches to the active sites of the enzyme is supposed to sit at the active site. So, this sitting phenomena is maybe like this some sit properly some split some you can slip like this. So, these phenomena we considered at the reversible phenomena this is cannot be irreversible.

But whatever enzyme whatever substrate, see that the active sites of the enzyme that gives the product. So, that is the reversible reaction this is what that you know Bricks and Helen they propose and this is how the reaction can be expressed this is E plus S equal to E S and E like this.

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Enzymatic reaction Kinetics

The rate of substrate consumption or product formation from Eq. (1) can be written as:

$$v = \frac{dP}{dt} = k_2[ES] \quad (2)$$

The rate of Enzyme-substrate complex formation can be given as :

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] \quad (3)$$

Since enzyme is not consumed, the conservation of enzyme yield [E] can be given as:

$$[E] = [E_0] - [ES] \quad (4)$$

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Now rate of the product formation, what is our equation that we have E plus S reversibly give E S am I right and this give irreversibly it gives a E plus P. So, this is k 1 and this is k minus 1 and this is k 2 now rate of product formation here this is plus P am I right rate of product formation this is the different rate of product formation is what that k 2 into E S this is k 2 into E S.

But rate of formation of E S is proportional to this is forward reaction this is backward reaction again this is degraded. So, it is k 1 into E into S and k minus 1 E S k 2 into E S. So, now, one thing we shall have to remember enzyme concentration any point of time E E 0 is the initial enzyme concentration equal to free enzyme plus bound enzyme this is the. So, E I concentration of be I can express that E 0 minus E S.

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Now, then when they when the Bricks and Helen try to judge justified this equation they have certain assumption that what they are saying when you plot here you see here plotted S by S 0 versus time.

The when this plot is like this then your this quasi steady state condition is valid and at the quasi steady state condition d E S by d t this should be equal to 0, but if the relationship is like this whatever what is shown here then your Michaelis Menten equation will know that positive steady state condition is not vary. Now what do you mean by quasi steady state condition, quasi steady state condition means tends to steady state it is not exactly steady straight, but the situation is going to the status that is called quasi steady state.

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Enzymatic reaction Kinetics

By applying quasi steady state assumption on Eq. 3, $\frac{d[ES]}{dt} = 0$

Therefore, $k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$

By rearranging, $[ES] = \frac{k_1[E][S]}{k_{-1}+k_2}$

From Eq. 4, $[ES] = \frac{k_1([E_0]-[ES])[S]}{k_{-1}+k_2}$

By rearranging, $[ES] = \frac{[E_0][S]}{\frac{k_{-1}+k_2}{k_1}+[S]}$;

Since, $v = k_2[ES]$, Therefore, $v = \frac{V_{max}[S]}{K_m+[S]}$; where $V_{max} = k_2[E_0]$ and $K_m = \frac{k_{-1}+k_2}{k_1}$

Now, this is finally, they have derived this equation and this is simple I hope you can all calculate this is shown here how it is done and what I wanted to want to point out this value k minus 1 plus k 2 by k 1 this is called this is equal to K m and an another thing that we have k 2 in to E S that is called V max this is basically this is called V max. So, I can write like this. So, this equation can be easily write in this form this is how we can write in this form and this is what V max equal to k 2 into E 0 and K m k minus 1 by like this.

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Enzymatic reaction Kinetics

The significance of K_M and V_{max}

- ✓ K_m is equal to substrate concentration when $V = V_{max}/2$
- ✓ Higher the value of K_m lower is the substrate affinity towards enzyme
- ✓ Lower the value of K_m , higher is the substrate affinity towards enzyme

Now, question comes how we can do what is the significance of the value of K m and V max because if you look at this equation V max S K m plus S am I right. So, what I can tell that V is directly proportional to V max. So, as the V max value increase the velocity of reaction increases and V is inversely proportional to K m the as K m increases velocity of reaction decreases. So, here what I have pointed out K m is equal to the substrate concentration because we have seen this is V versus S plot it is like this.

Now, here suppose this is the V max and this is V max by 2 now if you put this then you will find this is equal to K m value this is the if you if you V equal to V max by 2 if you put in this Michaelis Menten equation then you will find that S equal to K m. Now higher the value of K m lower the substrate affinity this is exactly how it is proportional and lower the value of K m higher the substrate affinity that is more velocity of the reaction. So, what does it mean they mean that suppose I want to produce a certain amount of product or if the K m value is low we required less amount of substrate, but if the K m value is high we require more amount of substrate there is the significance of this.

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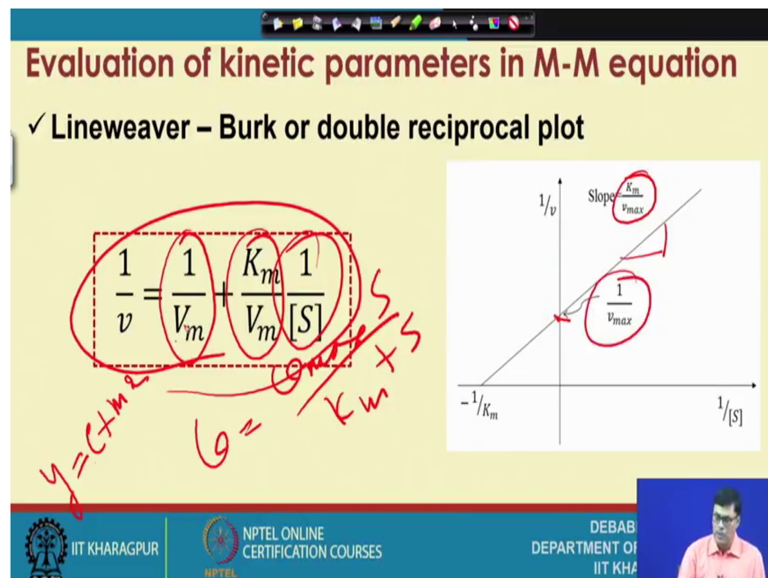

Now, question comes how we can determine the K m and V max value there are 3 different plots we have one is Lineweaver Burk plot another Eadie Hofstee plots and Hanes Woolf plot and this plots are very very simple money and that is the Lineweaver Burk plot is considered as the inverse relationship like we have Michaelis Menten

equation V equal to V max S K m plus S am I right now if you just inverse you will get this relationship just you impulse that you will get this. So, here you can see this is y this is 1 by V we can write y equal to this is constant V max is constant c and this is also constant this I m into and this is I can constantly m into x the y equal to m x plus c. So, it is the straight line plot, if we have this slope here and this will give you the value of K m by V max and intercept here will give you the 1 by V max value.

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Evaluation of kinetic parameters in M-M equation

✓ Eadie - Hoftsee plot

$$v = V_m - K_m \frac{v}{[S]}$$

Now similarly we can see that Eadie Hofstee plot I can write this Michaelis Menten equation in the form of this form we can write now if you write this form then V if you plot V versus V by S then what will this is negative slope. So, it is the negative slope will be there the slope will be if you take the slope those slope will be minus K m and this that this intercept if you look at this is started this is V. So, this maximum value we can consider the V max, directly we can find out the K m and V max value.

Now Hanes Woolf Plot we can write in other way.

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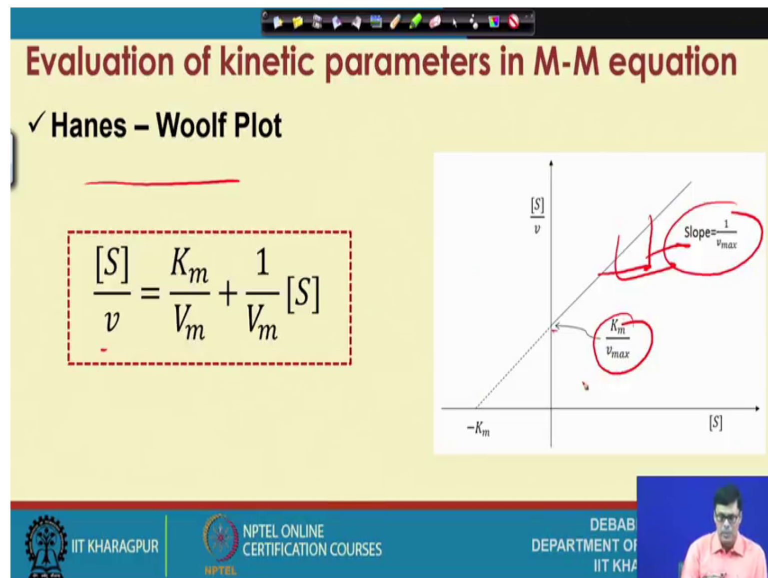

If we look at this is like this S by v equal to K m by V max 1 by V max into S. So, it is something similar to Lineweaver Burk plot it is also straight line plot only the thing is that this slope here slope will give you the value of 1 by V max and intercept will give you the V max by what is reverse in case of your Lineweaver Burk plot.

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Limitations of M-M Kinetics

✓ When S<<Km; $v \sim V_{max}$ S)/K_m,, it follows 1st order kinetics

✓ When S>>Km; $v \sim V_{max}$, it follows zero order kinetics

✓ V_{max} α [E_o]

✓ The enzymatic inhibition reactions are neglected.

✓ Does not consider multiple substrates

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So, question comes what are the limitations of the Michaelis Menten equation there are several limitations, I told you when I discussed the chemical reaction kinetics when you write any kind of mathematical equation we make a certain assumption and all the

assumptions are the limitation of this equation. I have given the simple example of that ideal gas law what is the ideal gas law p v equal to n r t and what is the limitation of this equation because this is valid for the ideal gas and what do you mean by ideal gas, ideal gas means it is imaginary gas this is nonexistence.

So, this is one thing you should remember another thing is that this Michaelis Menten equation does not take care the inhibition of that you know that they take a inhibition and another important thing is the Michaelis Menten equation considered 1 substrate 1 enzyme because if you look at the enzymatic reaction most of the enzymatic reaction they required the cofactor. So, basically it is 2 substrate and 1 enzyme molecule. So, in that way this does not explain that 2 substrate and 1 enzyme. So, these are the limitations of this Michaelis Menten equation.

So, in conclusion I want to tell that I try to explain you that what do you mean by enzymes I told you enzymes are basically their protein molecules what is protein is very nothing, but polymer of amino acids and protein has 2 different structure one is called guru buehler structure, another is the fibrous structure the that protein with the globular structure they are responsible for the formation of the active sites and a protein with fibrous structure they are not enzyme because they cannot form the active sites.

Now, the 2 type of enzymes we have one is the endoenzymes another is the exoenzymes some enzymes that Burk inside the cell some enzymes good out of the cell and work outside out of the cells. So, then I told you 4 different enzymes are there it can be classified into 6 different types and then I also explained the how does Hofstee, what is 3 different hypotheses, how substrate and enzyme they can interact with each other and give the product and finally, I discussed the Michaelis Menten equation, how Michaelis Menten propose the equation for rate of the enzymatic reaction and then later on Bricks and Helen they came forward to justify the Michaelis Menten equation with the help of reaction kinetics and I try to discuss what are the limitations of the Michaelis Menten equation and I also discussed what is the significance of the reaction constants K m and V max value and also right I also conclude that under that is the kind of limitations of this equation in addition to the what are the assumption we made for the Michaelis may do justify the Michaelis Menten equation. So, that is all for this lecture I think in the coming lecture I shall discuss about the inhibition of the enzymatic reaction which has a lot of application in the biochemical industry.

Thank you.