Course on Industrial Biotechnology
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Lecture 11
Enzymatic Reaction Kinetics

Today I am going to discuss.

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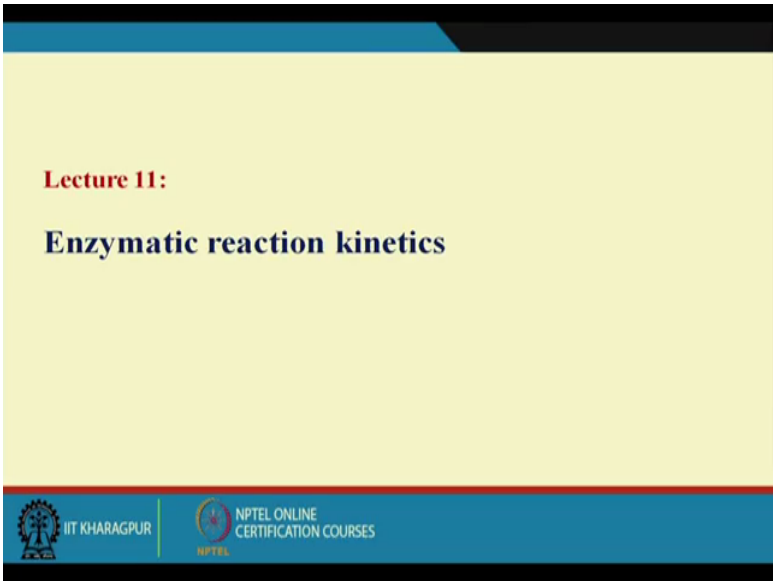

Enzymatic reaction kinetics. Now what is the importance of the enzymatic reaction kinetics? Because if you look at the that biological system I told you that mostly the reaction take place in presence of bio molecules. And this bio molecules may be active may be in inactive material. So but you know that as per as as a I am we are going to deal with mostly 2 type of materials 1 is living cell and non non living cell components.

And living cells they have the unique characteristics we calls they have the properties of reproduction. They are very sensitive to environment. Every living cell produce some kind of waste product and what all they are very sensitive to the environment and they are they have acclimatization property.

Well the enzymatic reactions enzymes are very specific 1 enzyme is very specific to 1 substrate, because 1 enzyme usually that they they does not participate in different reactions. As per example glucose isomer is acts on glucose and convert glucose to fructose and if you look at the microwave system microwave system we have metabolic pathways and each metabolic pathway if each steps we require 1 particular enzyme.

So we can we can consider the microwave system as a multi enzyme system. So to understand the microwave system first we should under understand the enzymatic system. So that is why I have taken this into account the enzymatic reaction kinetics.

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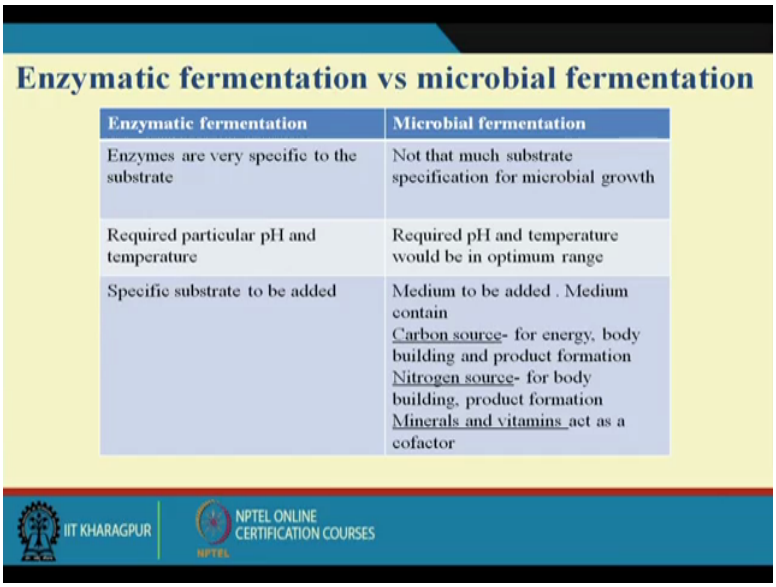

Now question comes how these enzymatic reaction form a enzymatic reaction differ from the microbial fermentation process? Enzymes are as I told you very specific to the substrate. Required particular ph and temperature and specific substrate to be needed but microbial fermentation process not that much substrate specific for the micro microbial growth.

As per example I can tell you glucose can produce ethanol it can produce the acetic acid it can produce citric acid it can produce the different type of product it can produce. But and it required ph and temperature would be optimum in range because every living system also there very since I told you that very sensitive to the environment so temperature and ph plays very crucial role and what all they required I told you also that it required carbon source, nitrogen source, minerals and vitamins.

Carbon they mostly required for the growth of the cells also a part goes for the source of energy. And 1 part goes for the product formation. Where nitrogen mostly used for the growth of the cells, minerals and vitamin they mostly used for the (())(3:44) carrying out as a co factor in the different enzymatic reactions.

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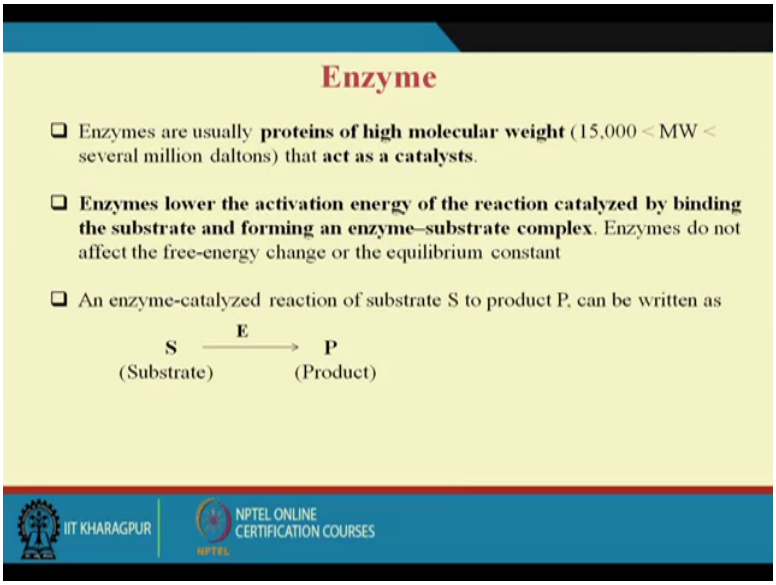

Now if you look at the enzymes. Enzymes basically they are protein molecule not necessarily protein molecule if you enzymes can be synthetically prepared but most games there protein in nature and they have global structure and global structure is kind of folded structure and during the this folding due to the hydrogen bonding between NH2 and C double O H.

The functional group with the R group of the different amino acids and during this folding they they they expose 1 ex the 1 layer 1 side that is 1 particular side which is very specific with the configuration of a particular substrate and that is why we call that protein with active side we call it enzymes. Protein without active side we call it inactive enzymes.

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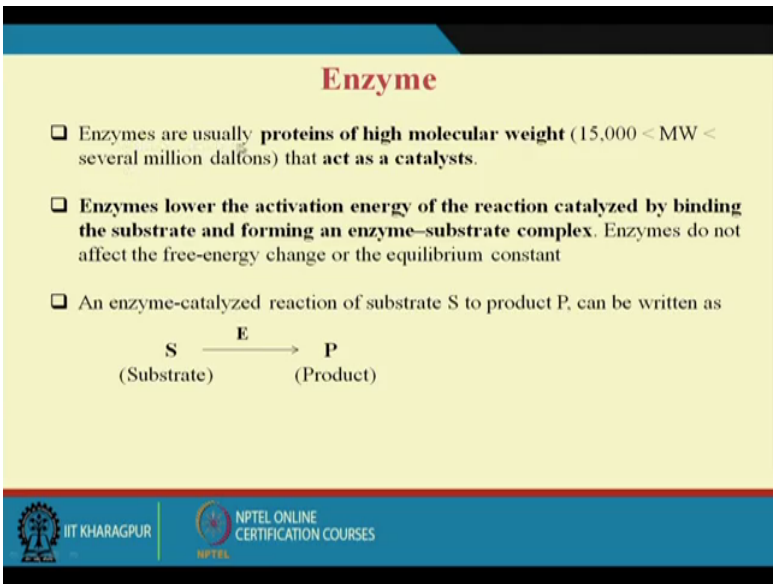

So here I want to point out that enzymes are usually the protein the high molecular weight with a act as a catalysts. And catalyst as you know catalyst main purpose of the catalyst is to reduce the activation energy and to accumulate the reaction. Enzymes lower the activation energy of the reaction catalyst by binding the substrate and forming enzyme substrate complex.

Enzymes do not affect the free energy change or equilibrium constant. Enzyme catalyzed reaction of substrate to product can be written as like this. This is a where this general form we can write like this.

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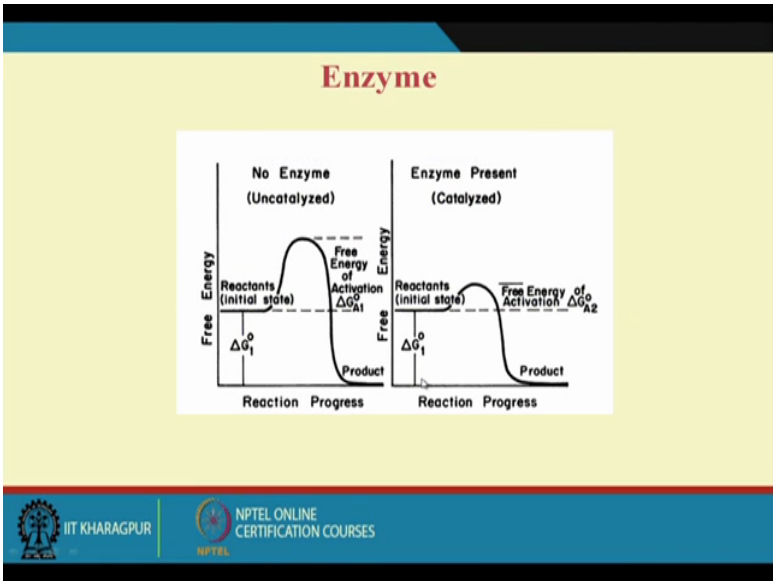

Now this is the how due is reduced. This is a this is a uncatalyzed process and this is catalyzed so what if an activation energy that is required that will be reduced because you see the how and that is how acc how the rate of reaction is accelerated. Another thing is that after the reaction is over the enzyme remain non alter because that is another thing that we have with the catalyst.

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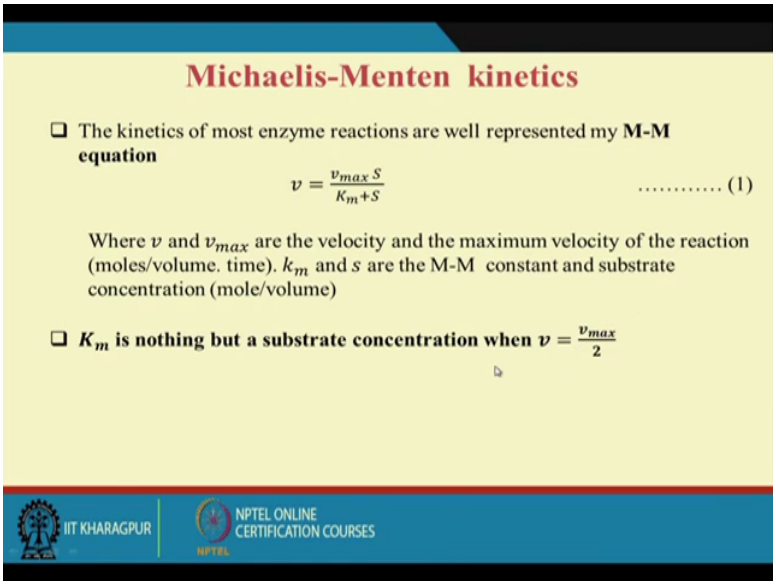

Now these enzymatic reactions usually explain by Michaelis menten with the very very famous equation they propose. Michaelis menten they propose the equation between V by S on the basis of the co relation between V by S as as given here.

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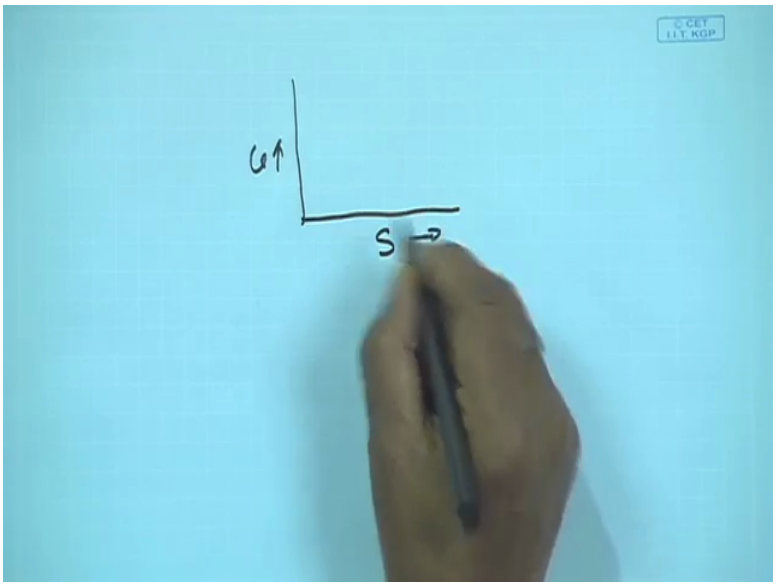

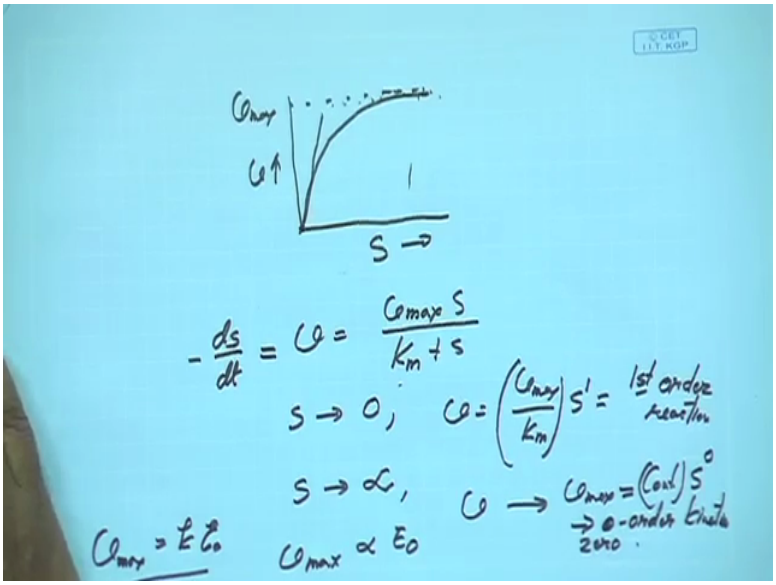

It is like this. This is the co relation that we have and what is the equation they propose V equal to V max is KM plus S. Where V is the velocity of reaction velocity of reaction I can express as minus DS by DT rate of substrate degradation and this is the substrate concentration and V max is the maximum velo maximum velocity of reaction this is V max and KM is the Michaelis menten constant.

So this is the equation that we have and Michaelis menten they proposed this equation on the basis of this co relation the question come the how how Michaelis menten proposed this equation because if you look at if S tends to 0 then what is happening it tends to 0 here then if S tends to 0 I can write V equal to V max KM into S. Tends 0 it doesn't mean it is 0 it is across to 0 though I can ignore as compare to this 1 so I can write now.

This is constant am I right? And if this is constant this is this is 1 so we this this is in first order reaction. First order reaction so at low substrate concentration it follow the first order reaction. Now when S is infinity S is infinity then what will happen? It comes here it is infinity then then the your reaction will be play to it will almost constant so when S is very high I can ignore KM and if we I ignore KM then I what I can write?

V tends to V max or I can write this is equal to constant into S to the power 0. S to the power 0 means it follow the 0 order 0 order kinetics. So at at high concentration it follow the 0 order kinetics 0 order kinetics. Okay. Now another thing that we have the V max is proportional to the free enzyme concentration. So this is equal to that is why the V max will be equal to what V max will be equal to K into E0 that is that is a so 3 assumption we have for this for this Michaelis menten on the basis of that they propose this equation. Now later on because.

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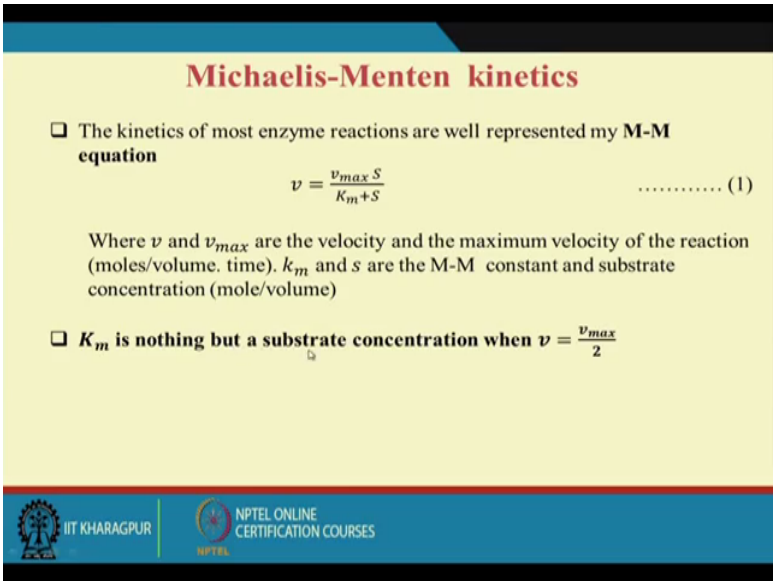

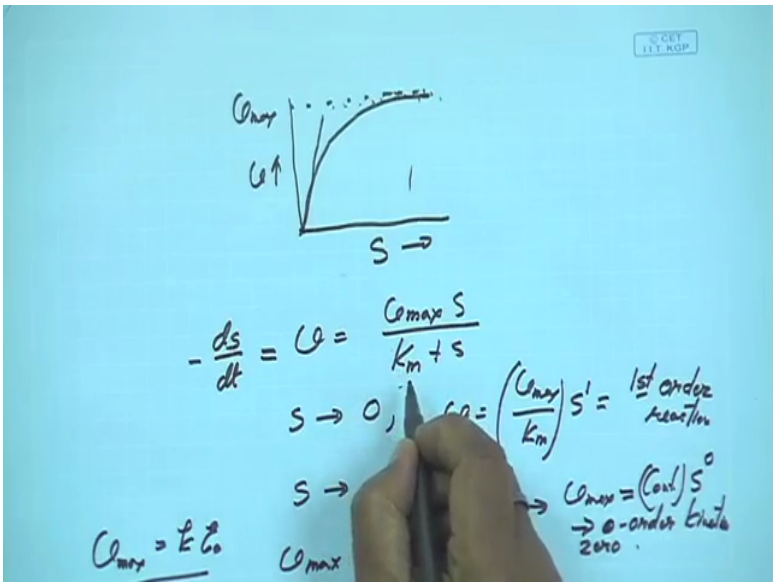

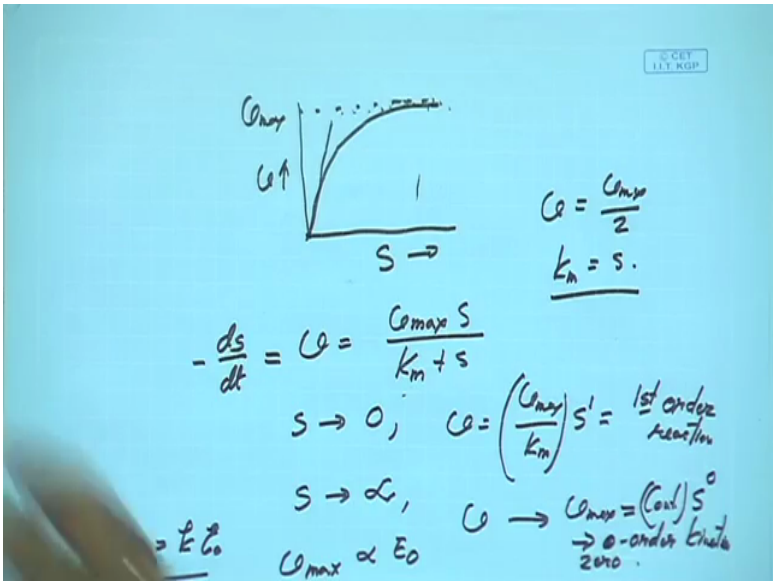

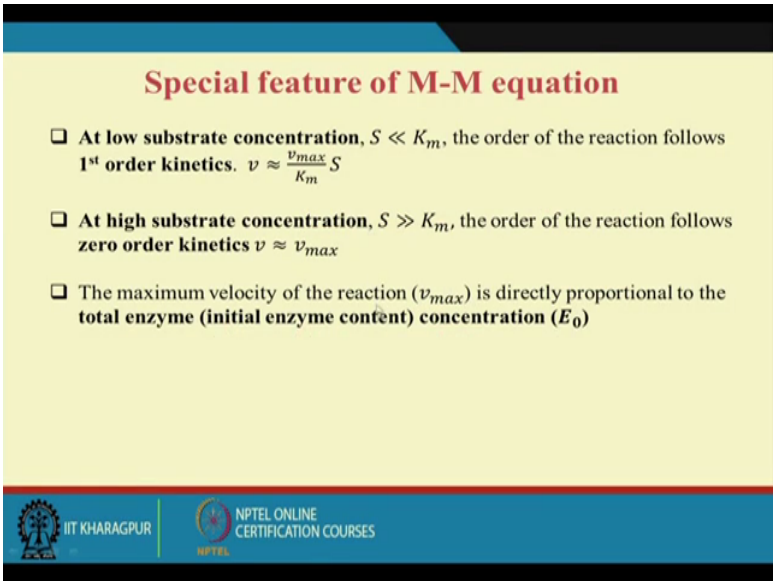

Now another thing we have written the KM came here KM is the very interesting. KM can be at V when V equal to V equal to V max by 2then KM will be equal to S. That is the substrate concentration now couple things we have that I have what I told it is written here at low substrate concentration it follow the first order kinetics.

Here the high substrate concentration is followed a 0 order kinetics and maximum velocity at reaction is proportional the free enzyme concentration and what is the maximum free enzyme concentration what is the initial enzyme content. The initially we have whatever enzyme that is the maximum enzyme concentration is proportional to that.

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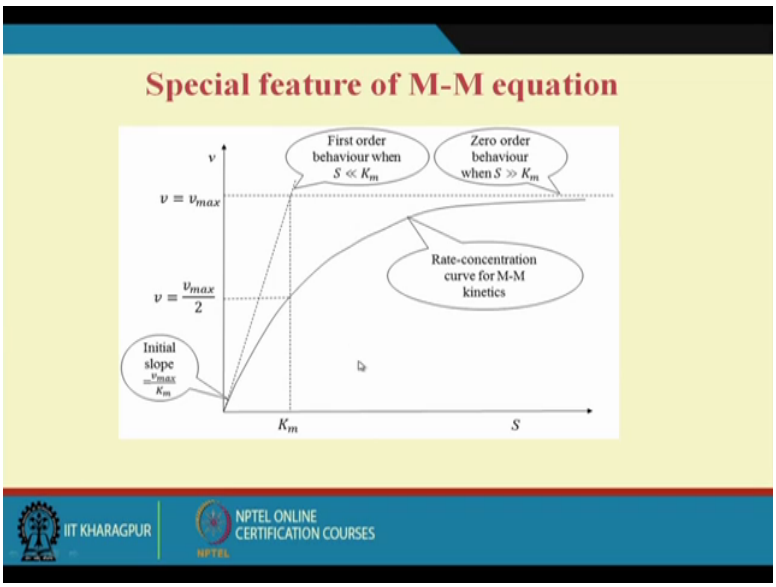

Now here at the the figure it is explained like this.

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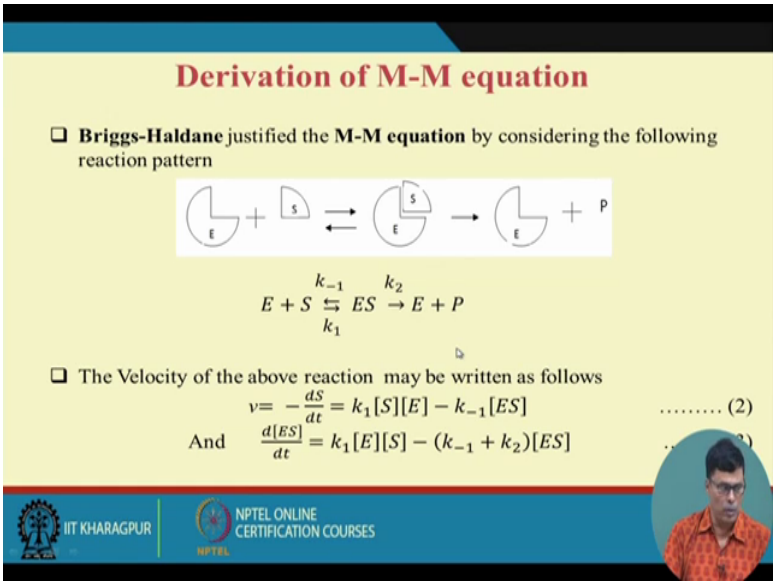

Now later on because two scientist one the Briggs-Haldane they justify the Michaelis Menten equation on the basis of some kinetics. What is the kinetics they have changed? That I told you protein that enzyme they have the active side now the substrate they will see that the active sign and when they try to see that active side this is this is a the some substrate can see properly some substrate can not see properly.

So that is why we consider the sitting phenomenon the reversible reaction. So they are saying here your enzyme plus substrate form the ES complex and this is reversible where something form ES complex something goes back to the original form but what if our enzyme substrate complex formation take place that gives the enzymes and product. So I can write like the equation format E plus S ES plus E plus P.

Now if we look at this is the rate of rate of degradation of substrate can be how we can write K1 into S, E into S into E minus K1 ES because it is going back this is reversible reaction so it is like this and rate of formation of ES can be written like this that you know that we have this is the rate of formation then it is going back this way and degraded this way. It is the form the product this way so it is coming like this.

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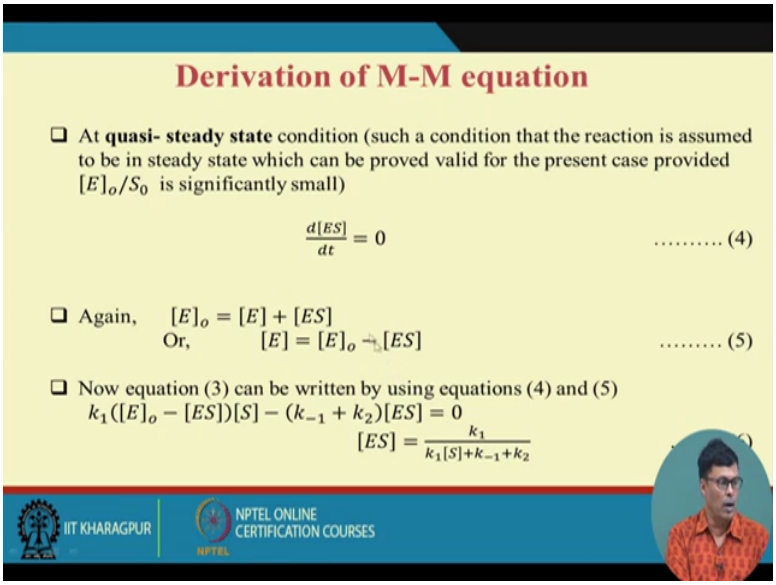

Now one when they assumed one quasi steady state condition now quasi steady state condition is very interesting.

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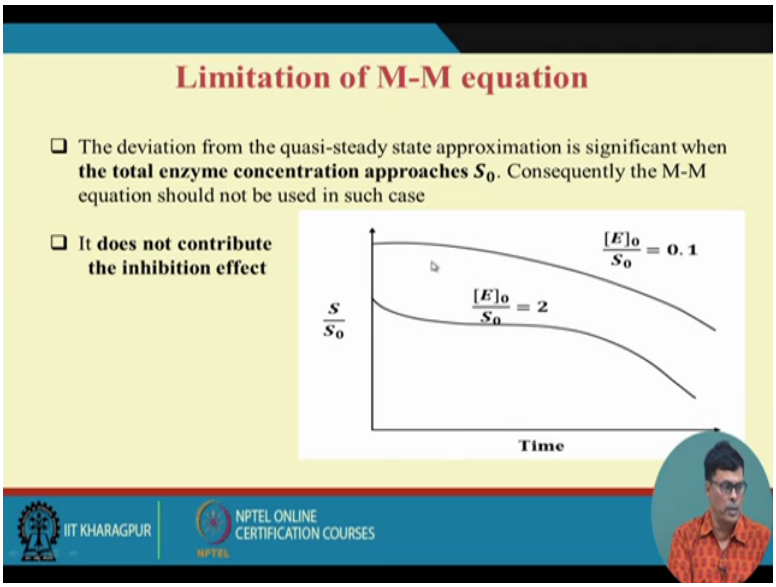

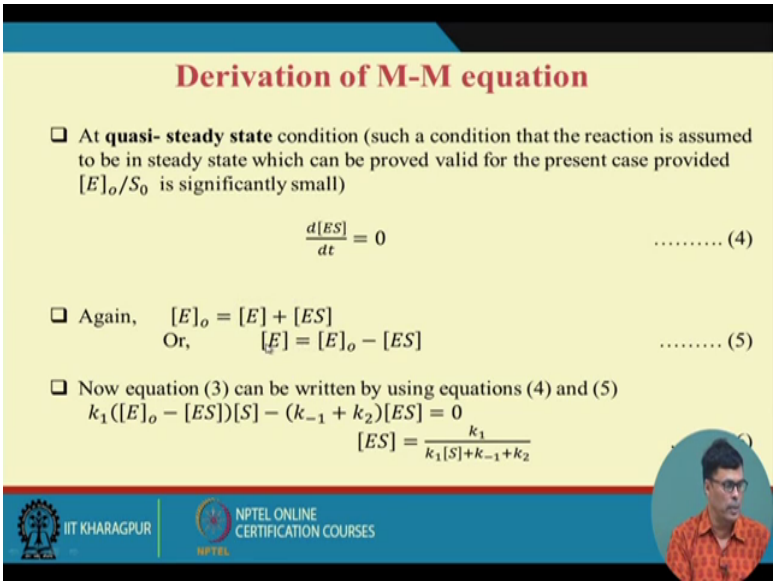

Here we can see that if we plot S by S 0 value what is S substrate concentration at time T. S0 is the initial substrate concentration now if S by S0 value is minimum then the profile will be like this and but as the E0 by S0 value increases it is deviating like this. It is it is not of this pattern.

Now now what briggs and Haldane they point out that this quasi state what is quasi steady state I told you is steady state condition what is that when there is the rate of forward reaction equal to rate of backward reaction and that is possible when you have E0 by S0 value is point 1 because you know that. But it is not that quasi steady state condition is tends to steady state not exactly steady state but approaching towards the steady state.

So this is the this is the thing that we have now here that E0 is at any point of time the total enzyme concentration equal to the free enzyme concentration plus bound enzyme concentration. Now if we write this rewrite this equation the equation 4 and 5 it will come in this form and finally we have ES equal to this.

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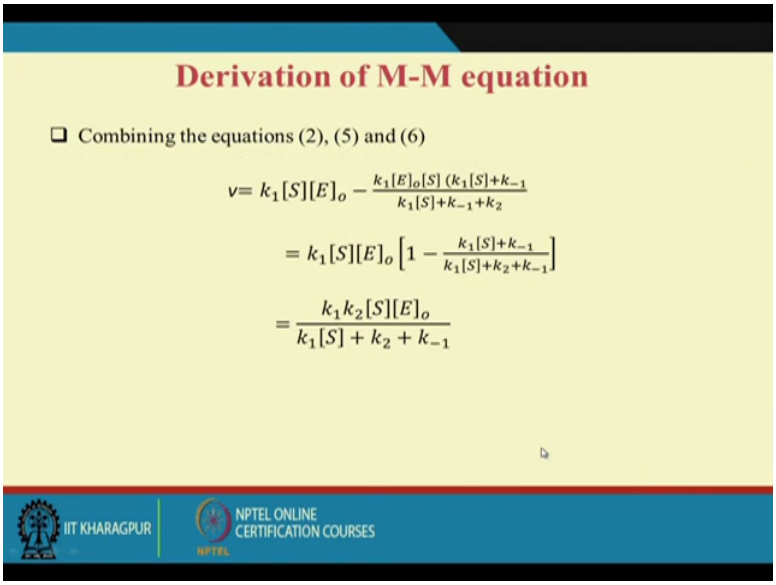

Then we have velocity of reaction equal to this if we put we can analyse then we can get this equation.

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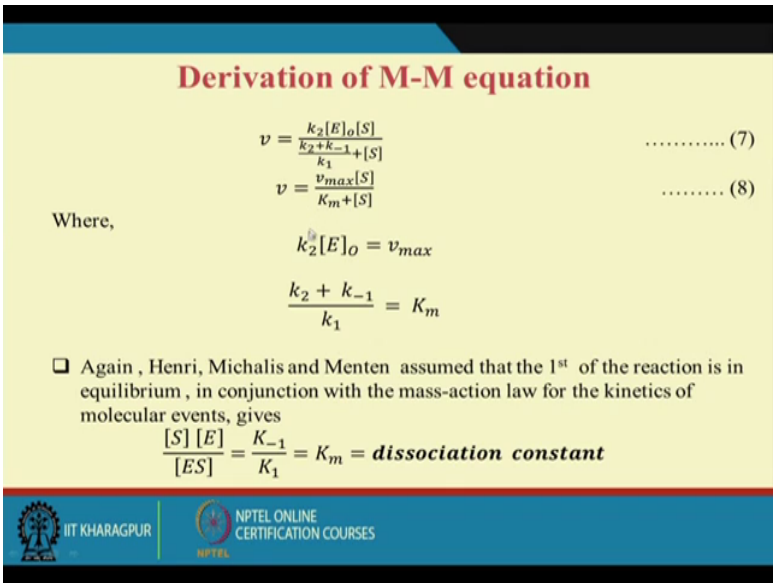

And finally we can write this equation in this format V equal to V max S Km plus S which is nothing but Michal that Michaels menten equation but it is possible when K2 when K2 into U 0 equal to V max and K2 mi plus K minus 1 by K1 equal to Km. So this is constant and this is also constant E0 is constant K2 also constant.

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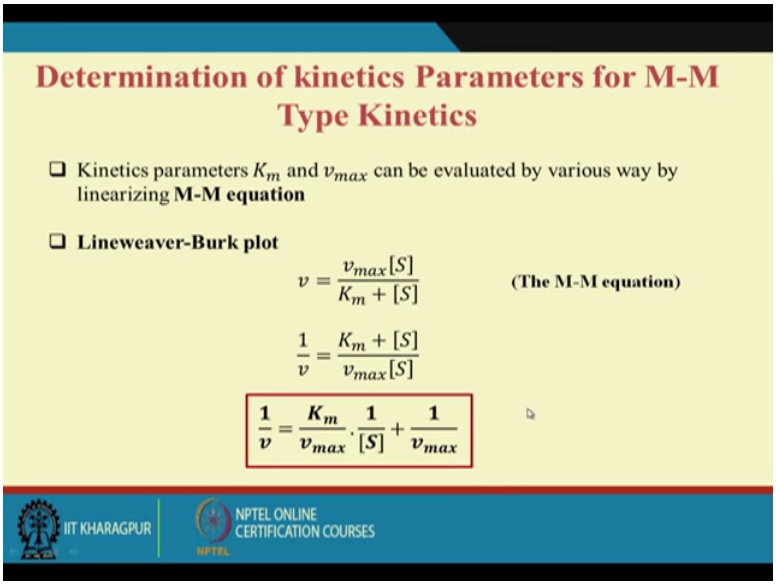

Now now question comes other points I want to point out here.

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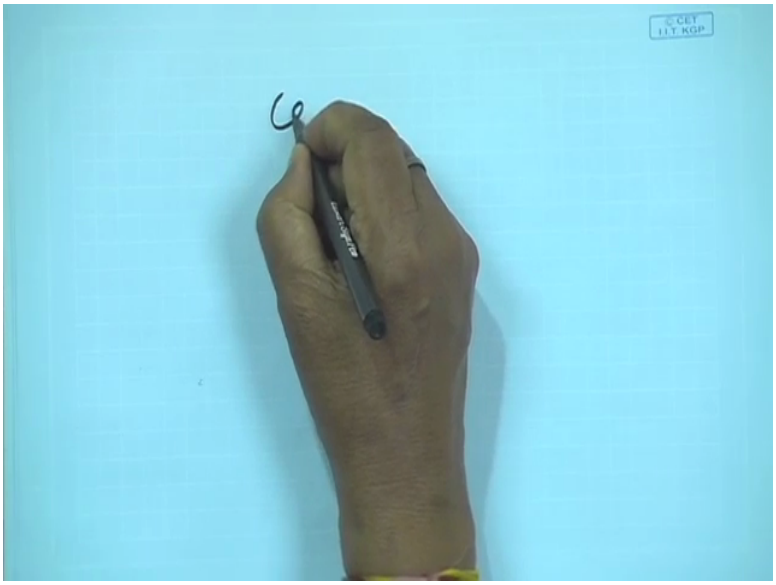

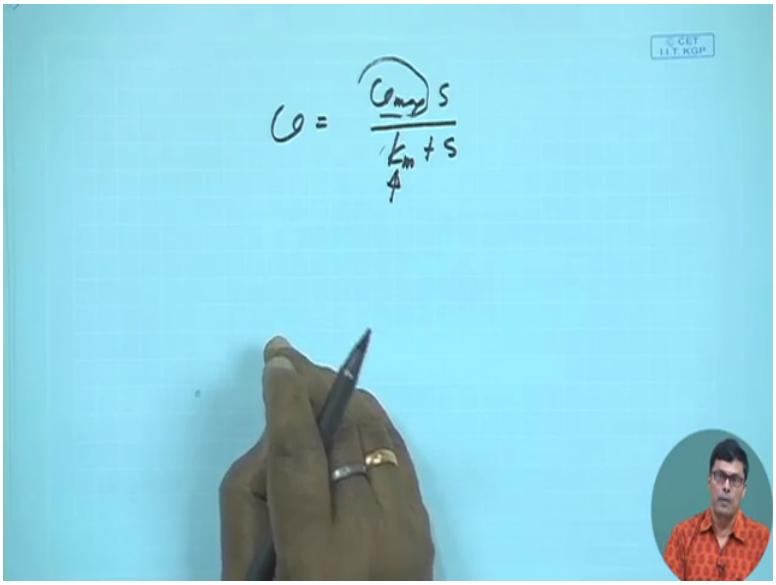

If you look at V equal to V max S Km plus S. Now what is the significance of the value of Km. Km significance is that Km indicates the affinity of substrate towards the enzyme if Km value if very high indicate that you required more substrate to get a desire amount of product but if Km value is low it indicate that your required less amount of substrate to get the desired amount of product.

So this is the significance of the value of Km and V max is if the if the red (()(15:12) axis high that your velocity of reaction will be high so what is the desirable thing is that V max should be for a particular enzyme V max should be as high as possible and Km should be as low as possible. And this indicates the kinetic constant of the enzymatic reaction.

Now question comes how this constant can be determined and what will be the unit of Km. Km will be same as the substrate concentration. Because Km plus substrate so whatever unit that we have for the substrate then we you need to should use for the Km. Now 3 different plots we can use to determine the value of kinetic constant Km and V max one is line order (())(16:00) plot. Another is hence and wolf plot. So for line order (())(16:08) plot is nothing but it is the inverse inverse relationship.

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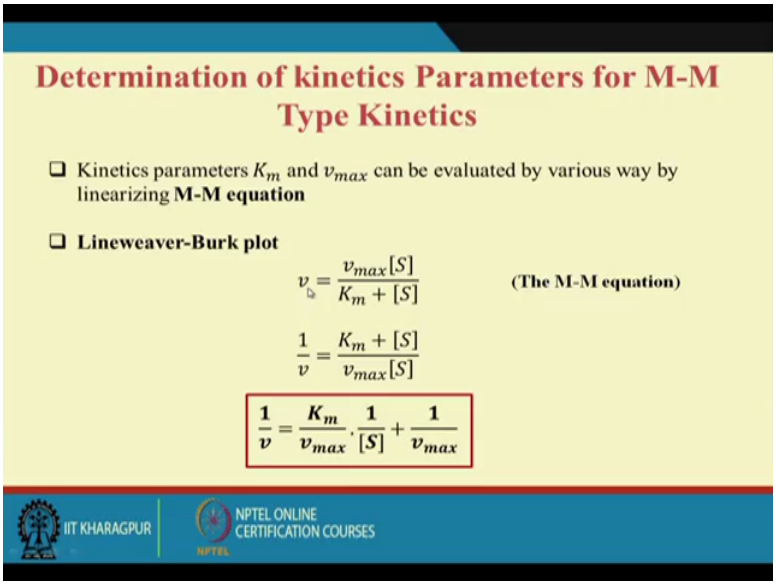

You see that V is equal to V max S KM plus S. And if you do the inverse 1 by V then then what will you get? That a KM plus S divide by V max S and 1 by V equal to KM plus Vmax 1 by S 1 by Vmax. Now it is very simple if you write 1 by V equal to KM Vmax 1 by S plus 1 by Vmax.

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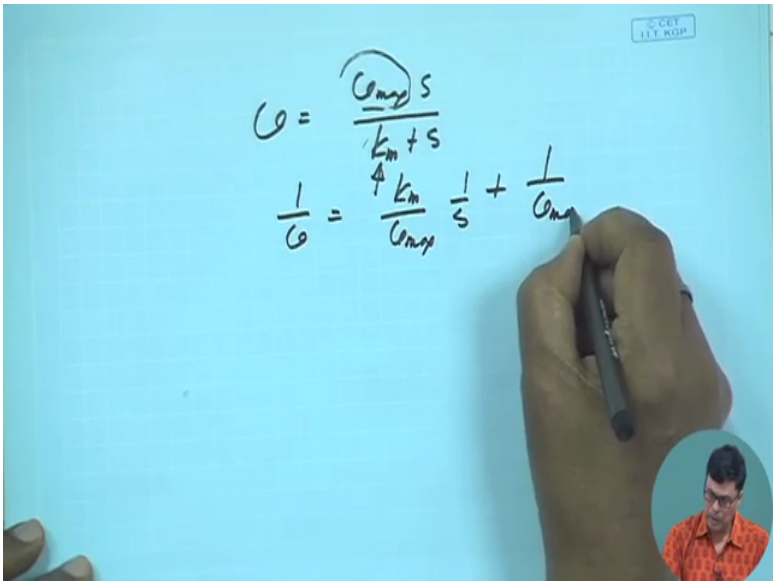

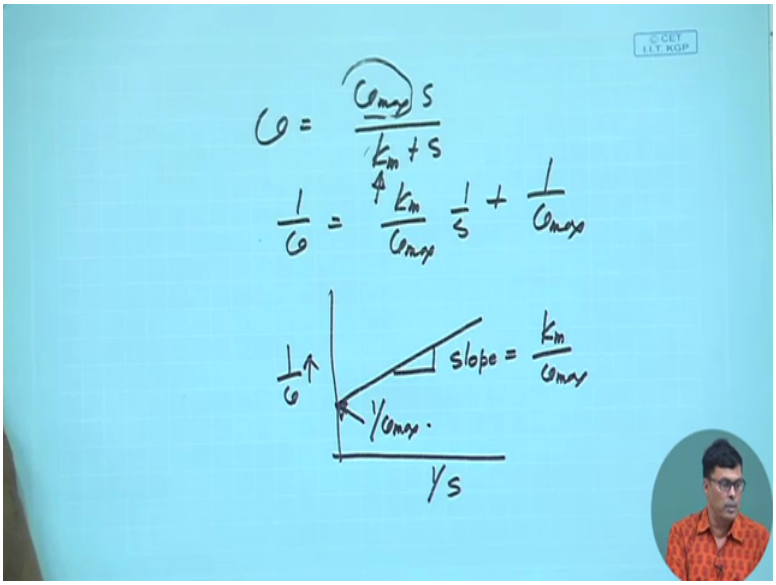

And then if we plot 1 by V versus 1 by S then we will get a straight line because this is a straight line equation. Slope will give you the value of KM by Vmax and intercept will give you the value of 1 by Vmax. Okay so this how we can find out? Now once we have this Vmax value we can put here and we can find out the value of KM. So so we can easily determine the Vmax and KM value of any enzymes.

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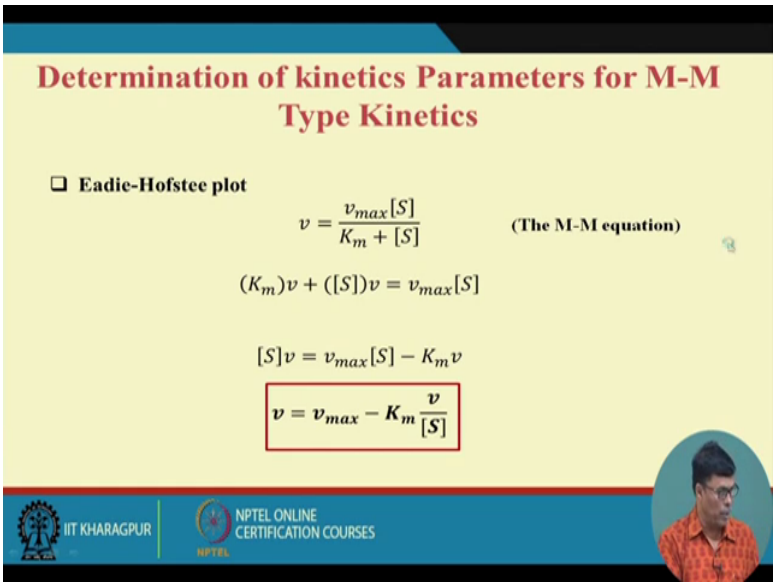

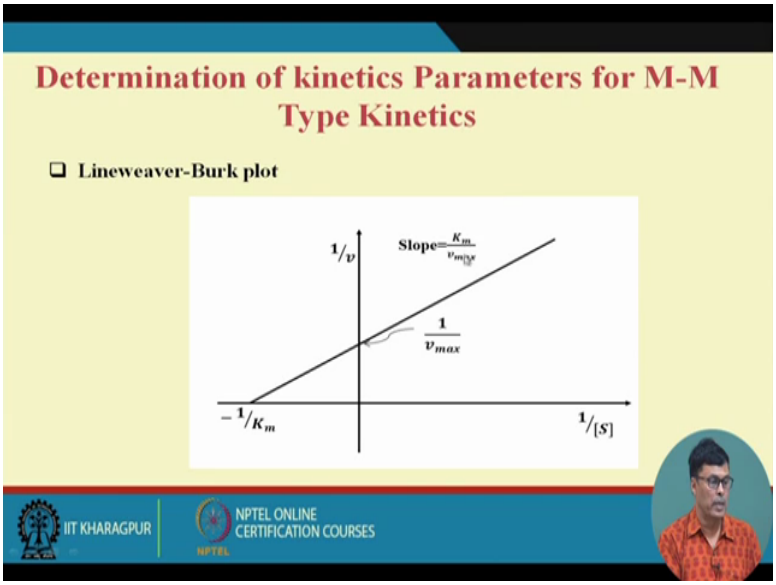

This is how it can be done? Now I can show you here that this slope is KM by Vmax and intercept is 1 by Vmax and here the intercept of the X axis here what will happen 1 by V equal to 0 and if you put 1 by V equal to 0 then we will find 1 by KM is minus 1 by KM is equal to 1 by S.

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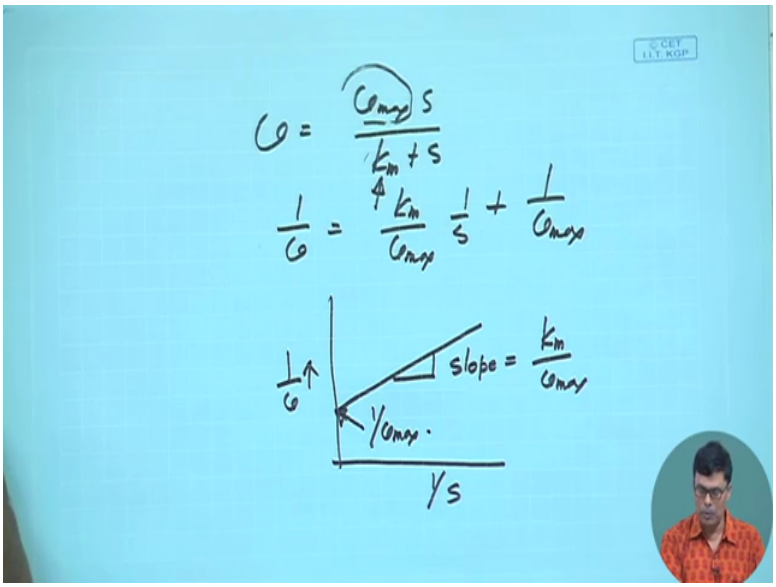

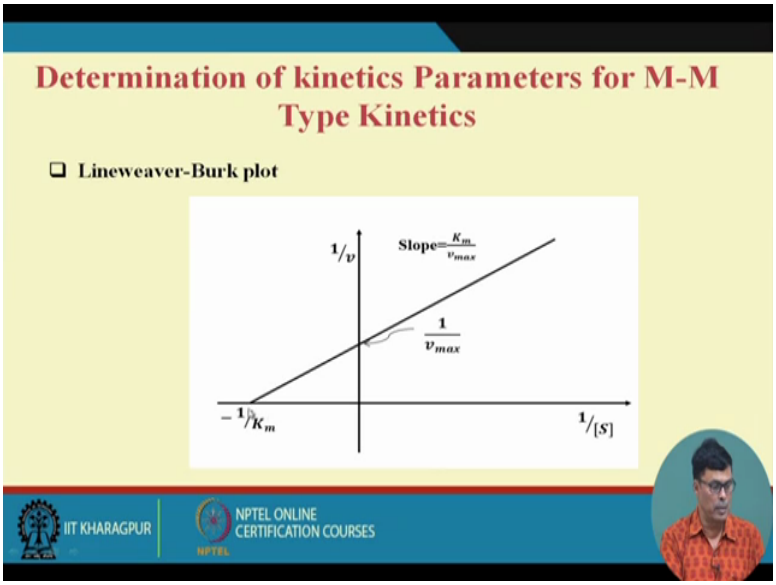

So that you know that this is equal to that. So you can from the intercept also you can easily find out the value of KM.

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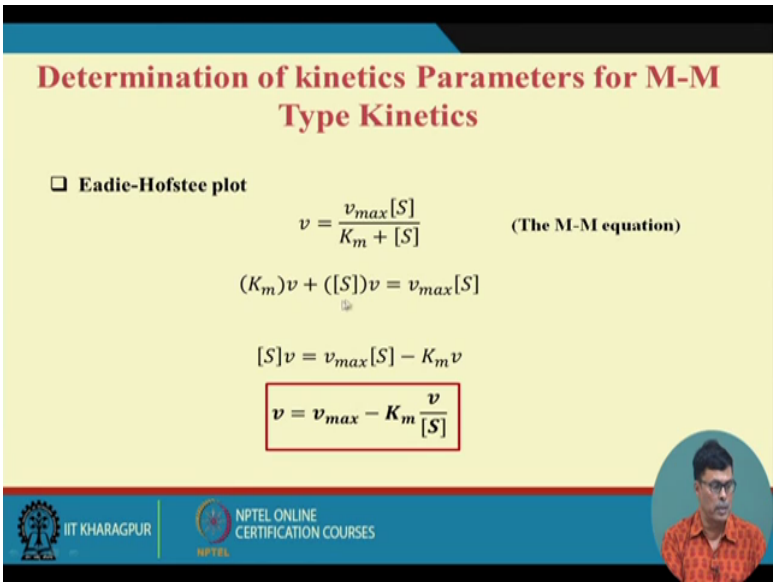

Now this is eadie- hofstee plot. Eadie-hosfstee plot is the like the there is same equation and we ultimately come of this. This is V equal to Vmax KM V by S.

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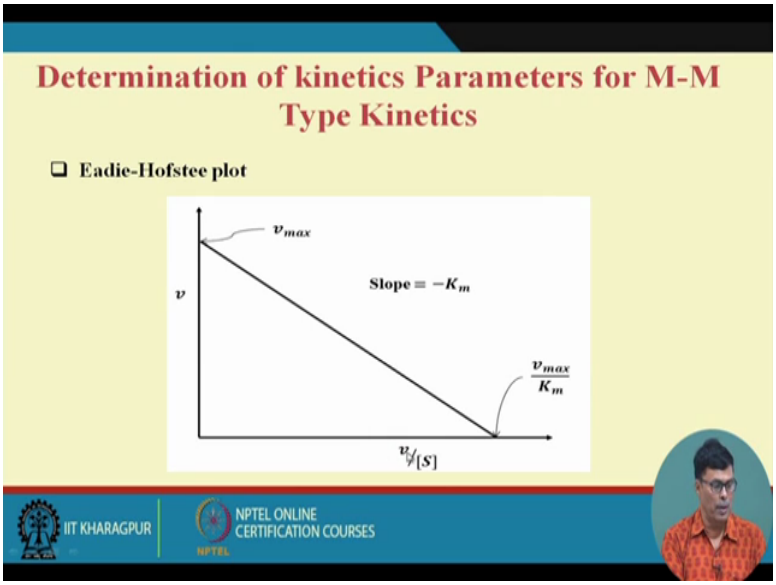

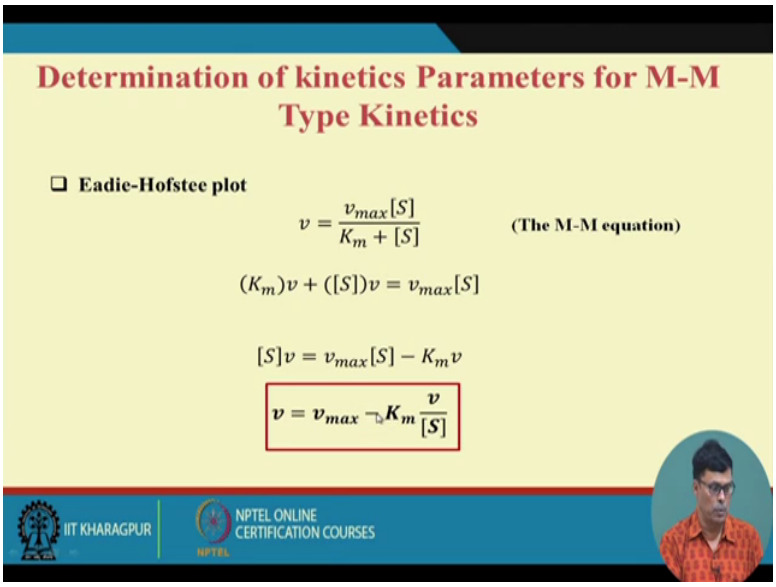

Now if you plot a V by S then it will negative. If you go back that it is negative slope. Am I right? This is negative slope so you will get a negative slope here. And this this intercept the interesting thing is that here here the this this this value is what this value is Vmax V by V by S this is a Vmax and slope will be the value of KM so we can directly find out the value of Vmax and KM the KM directly you can find out from the slope here and from the intercept you can find out the Vmax value.

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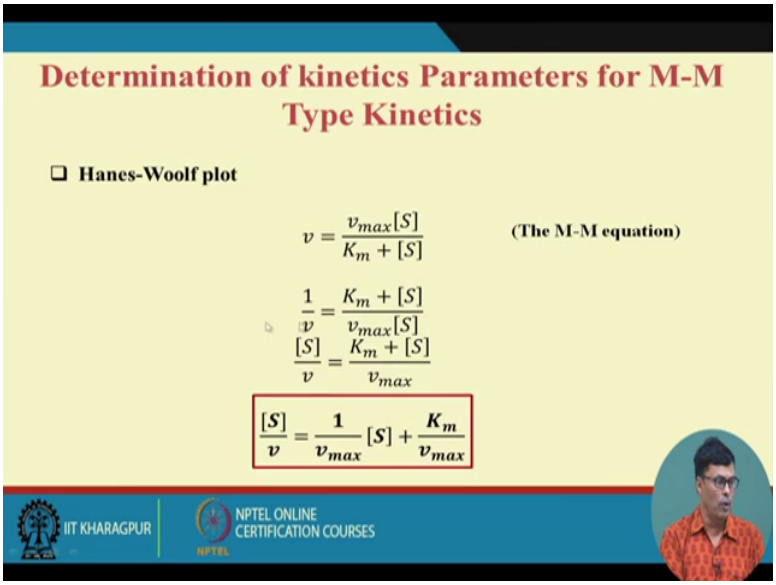

Another plot we can plot what you called hanes and woolf plot this is the S by V 1 by Vmax is so it is also similar to your straight line equation you see this is constant and this is also constant and this is the variables and S is the variables.

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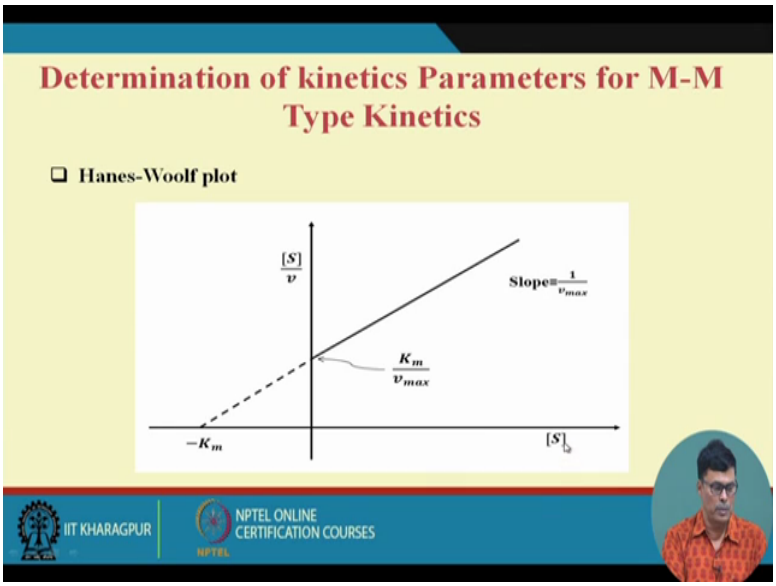

Now if you if you plot S by V versus S then intercept will be Km by Vmax and slope will be 1 by Vmax. So Vmax value you put it here you can find out the KM value so any of this plot can be used for finding out the kinetic constant of a enzymatic reaction.

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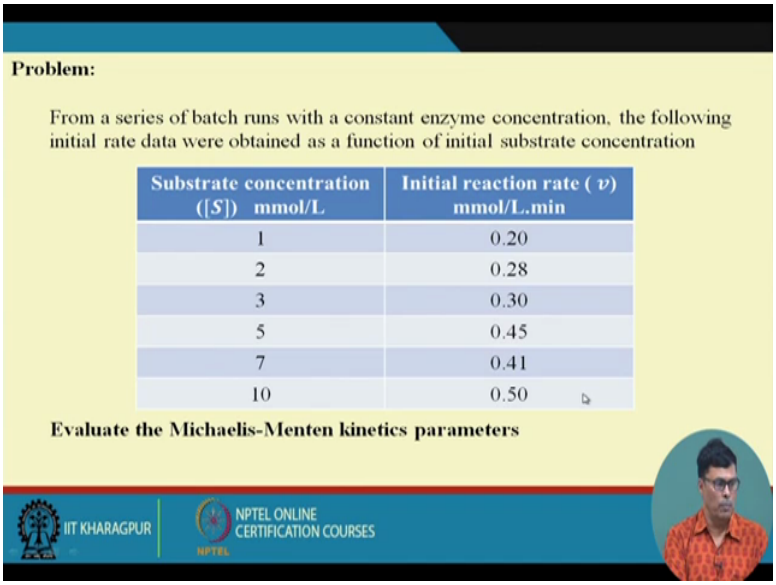

Now that that you know that this problem that let us have a have a problem how how we can really solve this problem.

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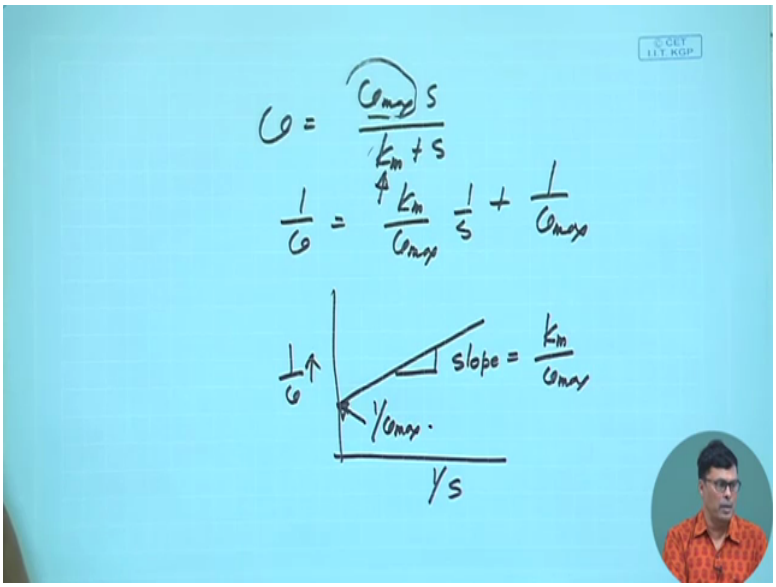

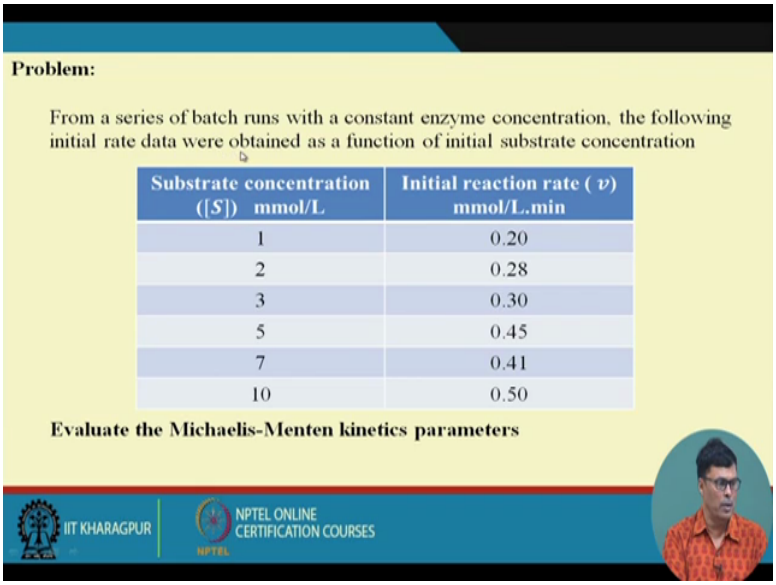

From a series of batch run with a constant enzyme concentration the following initial rate data where obtained as a function of initial substrate concentration. So as the substrate concentration initial substrate concentration increases your rate, we observed at the different. So evaluate the Michaelis- Menten the kinetic parameters.

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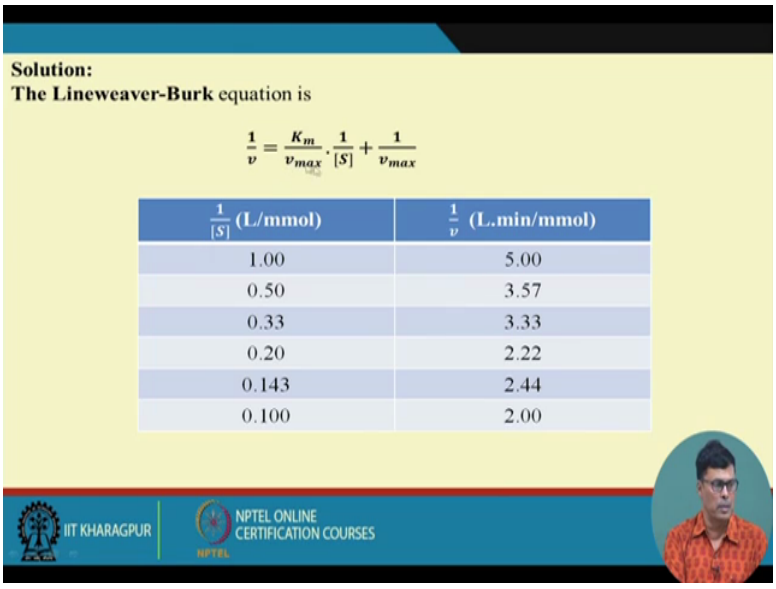

Now this is this is a lineweaver equation that we have already derived so if you look look at this equation this table and from this table this is S this is V. So we can write to 1 by S by 1 by V value. So 1 by S by 1 by V value we can write that and then we can plot .

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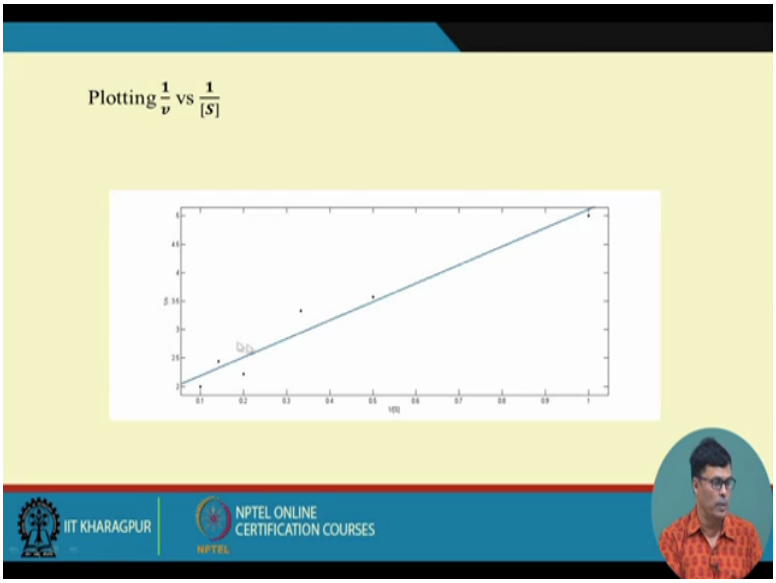

We can plot 1 by V versus 1 by S and we have will get stratight line and from the slope will get the the KM by Vmax and intercept will give you the value of Vmax and then we can calculate we find the Vmax equal to point 54 minimoles per litre per minutes and KM equal to 1.75 minimoles per litre. So this is how we can we can now couple of interesting thing we have enzyme that enzymes or or or very sensitive to the temperature also.

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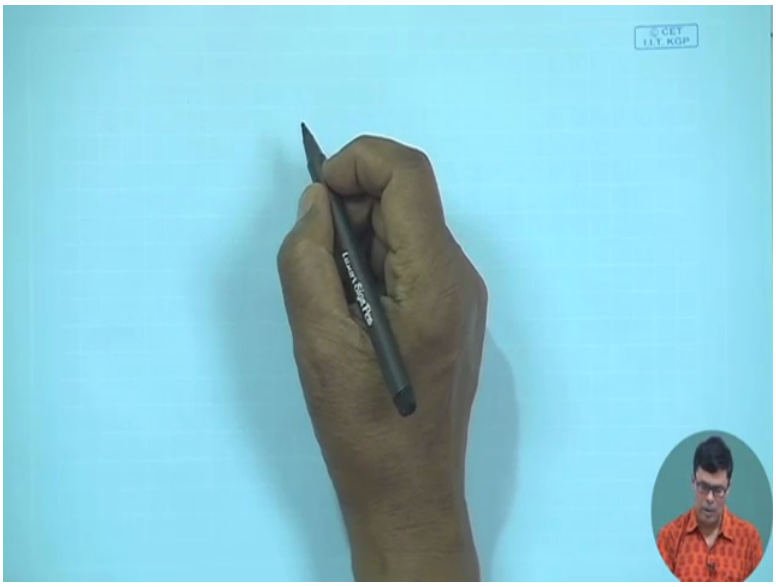

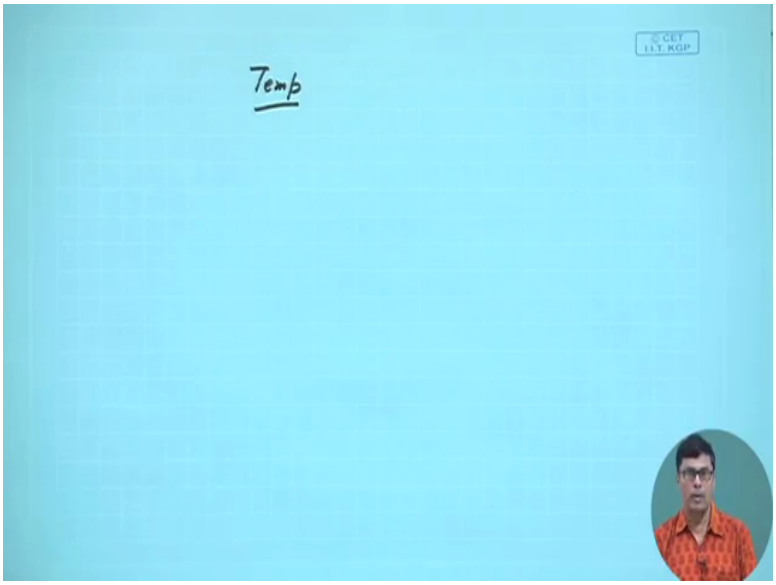

As you increase the temperature what will happened that that you know I told you that that enzymes are basically the protein molecule and they have the global structure and this globular structure the folding nature of this protein and this folding unit is due to the hydrogen bonding. Now when we increase the temperature that we give some kind of heat energy and hydrogen bond have some energy.

It is usually the 732 327 the kilo cals per mole. Now if you increase this heat then what will happen solution the hydrogen ball will be loosened? And when time will come when it will be totally oldest structure will be unfold when when it unfolded then enzyme will losses activity.

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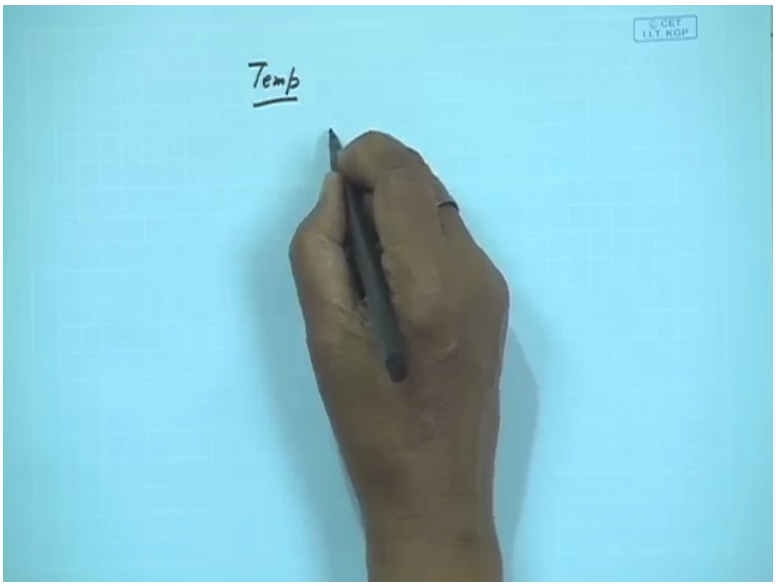
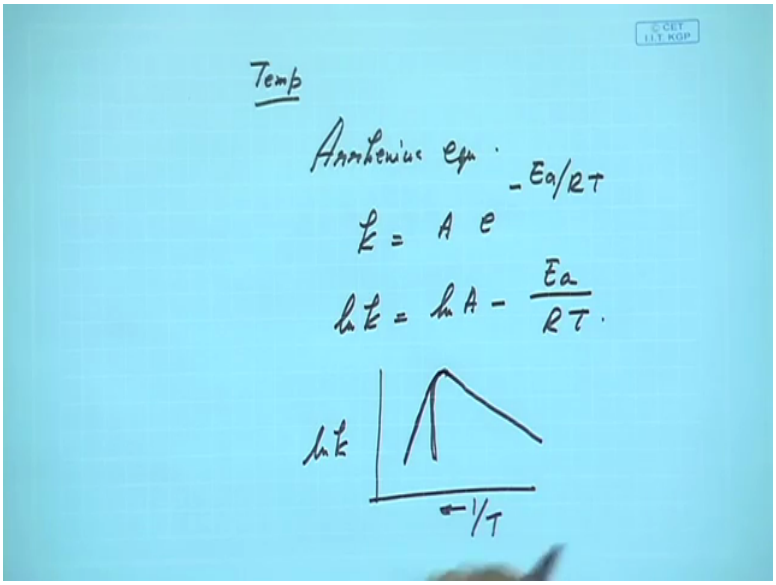
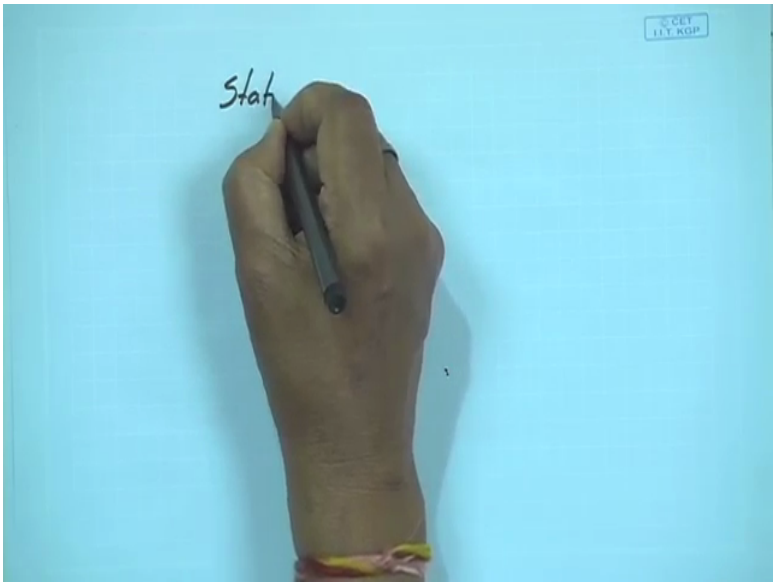



© CET
I.I.T. KGP
Temp
Arrhenius eqn.
$k = A e^{-Ea/RT}$
$\ln k = \ln A - \frac{Ea}{RT}$.
ln k
1/T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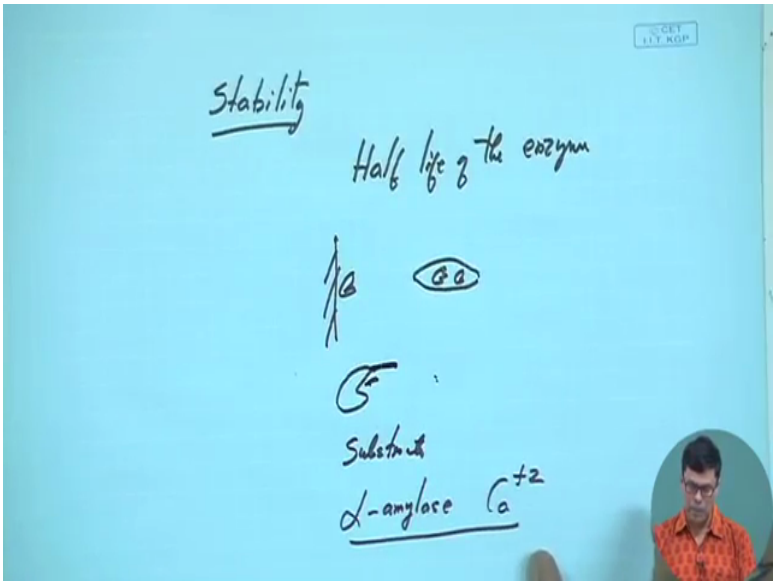
Stab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Stability
Half life of the enzym
Ea
Substrate
α-amylase
Ca^{+2}

Now for now we know that we have a correlation that what you called Arrhenius equation then K equal to A e to the power minus Ea by RT. Am I write? Now if we plot if you take log L and K then L and A minus Ea by RT. Now if you plot here the semi lock paper L and K by 1 by T. So you will get a slope like this. And then it will fall down like this. Now here I want to tell you something that because I told you the enzyme they are bio they are catalyst.

And they are basically bio catalyst. And we have several reaction in the in the in the chemical engineering which is carried out by also different catalyst like platinum paledium nickel they access the catalyst. Now how those enzymatic this catalytic reaction differ from enzymatic reaction. Now this enzymatic reaction as you increase the temperature when you are you are reporting molecules unfold.

Your your this fault will be very(())(24:14). Because you know that because when is the(()) (24:17) it is the universal phenomenon it is not a reversible phenomenon. But in case of inorganic catalyst this this this we even you increase the temperature this is the increase of temperature. 1by T decreases means T increases so as as as you increase the temperature then slowly slowly activity of the catalyst will go down and it decrease like this.

This is how it different from from enzymatic reaction and the chemical catalytic reaction. This is how they differ from it. Another very interesting thing when we go to the market we want to purchase the enzymes we always concern about the life. What is the stability of the enzymes? That plays very important role. Now how you because how you how you find out the stability of the enzyme?

Stability of the enzymes we determine with reflects to the half life of the enzyme. Half life of the enzyme now if the half life of the enzyme is more that means stability of the enzyme is more. Now naturally we know the proteins are costly and protein with active side we call it is enzymes. So they that will more costly because this is the active enzymes active proteins and so question comes that stability of enzymes that plays very important role.

And question comes how we increase this stability of the enzyme. The enzyme stability can be increased 3 different ways 1 is by immobilization of the enzymes. If we if we have suppose there is solid matrix this is the solid matrix and you immobilize the enzyme on the solid matrix or you have membrane inside the membrane if you store your enzymes.

So you can there is different techniques of immobilization that I self discuss because immobilization technique has several advantages and that is used by the industry. So different

way this can be it can be the stability can be improved. Second approach is that that enzyme with active side.

Suppose this is a active side and if we have some kind of polymer of amino acid, poly (()) (26:43) we have fine that it is informed this close to the active side keep some kind of protection to the active side another is the substrate analogue. If you have some kind of substrate analogue or some kind of presence of some kind of metal ion, we can increase the stability.

I can give the example of alpha mi lense alpha mi lense enzyme alpha mi lense enzyme in presence of calcium ion stability of the enzyme increases to a great extend so like this you know that stability so this plays very important role. So in conclusion what I want to tell that that that enzymes they are globular protein mostly they are globular protein they have the active sides.

And and the enzymatic reaction can be explain with the help of Michaelis menten equation and Michaelis menten they propose this equation on the basis of co relation between Vmax and S. But later on the eadie- hofstee they justify the Michaelis menten equation with the help of reaction kinetics.

But what we say that that substrate that see that the active sides and this is the reversible phenomenon something see properly something can not be sit in properly that loose a slip out and but you know whatever enzyme substrate they sit properly they give the product that is the reversible reaction on the base this is the this justify the Michaelis menten equation.

And then we have seen that a that enzymes they are very sensitive to the temperature as the high temperature it protein undergo the folding structure will be unfolded that whole activity will be loss and the has stability of the enzyme plays very important role and this is determine from the half life of the enzyme. Thank you very much!