Aspects of Biochemical Engineering
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Lecture - 24
Kinetics of Enzyme Catalyzed Reactions Using Free Enzymes – IV

Welcome back to my course Aspects of Biochemical Engineering. Now we have we are presently we are discussing very important topic that is on enzymatic reaction kinetics. In last couple of lectures, I try to concentrate on the how what is what do we mean by enzymes, how the activity of the enzymes can be expressed, and how the substrate and enzyme they can interact with each other and give the product, and Michaelis Menten equation just to find out the reaction kinetics, and how bricks and Hellen justified the Michaelis Menten equation with the help of reaction kinetics that.

Now, Michaelis Menten actually that the proposed the equation v equal to v max s by km plus s on the basis of correlation between velocity of reaction and substrate concentration and later on bricks, and Hellen justified this with the help of reaction kinetics. Now, but then we try to find out how the different constant that v max and km can be estimated with the help of different plots like live Lineweaver-Burk plot Eadie-Hofstee plot and Hanes-Woolf plot.

Now, then we also discussed that how substrate and enzyme they can interact with each other to give the product there are different hypothesis, lock and key hypothesis proximity effect orientation effect. And then we develop the correlation mathematical correlation between substrate concentration and time of reaction just to find out that at what time will get the how much substrate will be converted, then we also try to find out that different type of reactor we considered like batch process CSTR continual starting reactor or plug flow reactor, how the time batch time or space time for the CSTR and the plug flow reactor can be determined. Also we discussed some problems how this this help you for determining the volume of the reactor and on what basis you can tell that what should be the proper design for getting a desired amount of product.

So, this this way you covered in the last couple of lectures, now today we also we are going to discuss a very important topic that that enzymes are very sensitive to the environment. So, different physical chemical parameters plays very important role or on

these enzymatic activities, and as physical parameters when we considered there are three different parameters we have one is temperature shear forces and surface tension, now when you talk about the chemical parameters mostly we concentrate on pH and the pH is the nothing, but concentration of hydrogen ion in the reaction mixture and the concentration of different chemicals present in the reaction mixture.

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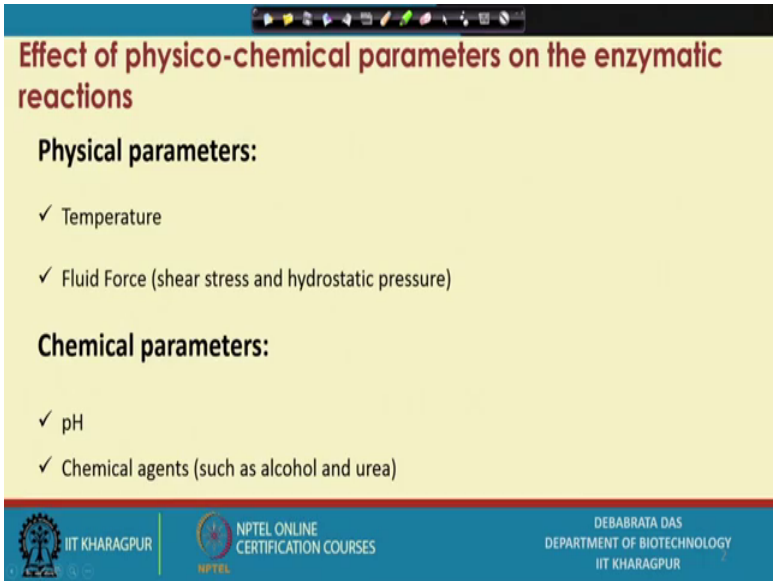

The first I want to discuss that the defect of physical parameters. Now if you look at the physical parameters we have temperature, we have temperature, then we have fluid forces and also I told you the surface tension. Now first let me give you that force is the effect of this temperature.

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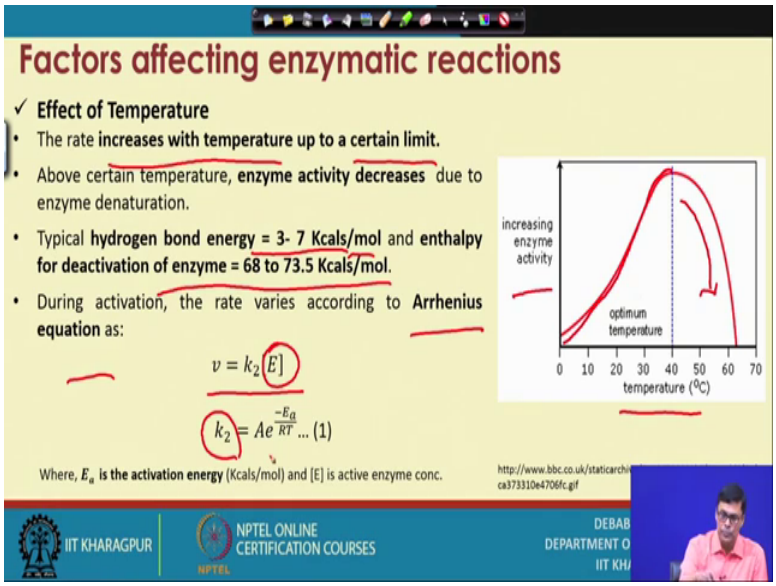

Now when we talk about temperature that that the rate of reaction, increases with temperature am I right with the under so up to certain limit. Now question comes with the increase of temperature why the rate of reaction increases that is the question.

Now, if you look at the answer to this question is this, that you know I already mentioned that enzymes mostly their protein in nature. And protein this proteins are mostly they have the folder structure, and this folder structure is mostly due to the hydrogen bonding. Now if you look at the strength of hydrogen bonding is very less 3 to 7 kilo cals here it is it is given here you said 3 to 7 kilo cals per mole and, but if you look at the deactivation energy of the enzyme is about 68 to 73 kilo cals per mole. So, it is quite high.

So, now if you increase the temperature; that means, you are putting some energy to this particular mixture. So, enzymes a time will come that this force that force of attraction due to hydrogen bonding that will be totally nullified. And when it is nullified the enzyme will be unfolded and well as soon as this is unfolded, the active site that is formed in say in the enzyme that will be lost as soon as the active site loss then the enzyme with loss is activated. So, this is how temperature affect this this activity of the enzyme.

Now, position comes the initial phase if you look at here if you the typical example, this is the enzymatic activity that the temperature. As the temperature increases this is slowly slowly increases, then it is all very rapidly. When it is unfolded then actually that the

activity of the enzymes is fall very rapidly because when the on pole and this is the irreversible in nature this is not reversible in nature. So, this is the major constraint now question come as you increase the temperature why the enzymatic activity increases. The reason is possible explanation to this is that, that you know since it is the hydrogen bonding and you are putting more energy. So, it as you increase the energy you keep the heat energy. So, if the your active site will just you know that it is about to unfold that kind of oscillation characteristics that will be build up, and with that characteristic more substrate will be attracted to the active site and give the product.

This is how the velocity of reaction increases with temperature. Now the decrease I explained that due to the denaturation of protein how the decrease is taking place in this way. And now during the activation period this equation is the v equal to k into E that is the velocity of reaction depends on the enzyme concentration. Now this velocity of reaction is as per Arrhenius equation this is the Arrhenius equation A equal to E e to the power E a by RT.

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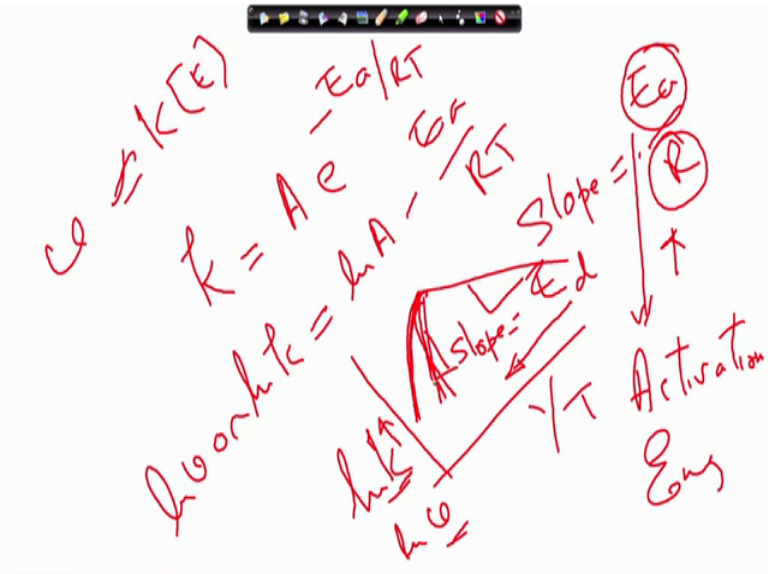

Now if you look at, when you write the Arrhenius equation k equal to A E to the power minus E a by RT am I right.

Now, and that k we have already shown the k is equal to k into E. We have we have shown that. So, it can be it is proportional to v am I right. So, I can write l n k or I can write or l n v this is equal to l n A minus E a by RT I can write it like this. Now if you

plot that l n k or l n v versus 1 by T. So, you will be having this kind of plot. So, if you when 1 by T decreases; that means, T increases here k value increases or if you write l n v velocity of reaction increases, but when it falls this falls very solved. Because this will if you are not here I want to mention that we have we know that inorganic catalysts. Inorganic catalysts also that you know, but they are here they are also at high temperature deactivation take place, but their deactivation nature is little bit different like this they are not solved.

Here the in case of enzyme this fall will be very short, but in case of inorganic catalysts like palladium, nickel this fall will be little bit slow you know that as compared to that of the reason is that in case of enzymatic reaction the here the total unfold of the that you know protein molecule will take place. So, that your enzyme will loss the total activities. Now here if you take a slope here, what will be the slope? Slope will be equal to E a by R T am I R am I right. The R value is the R is the gas constant now if you put the value of r you can easily find out the E a value what is the E a value? E a value is the is nothing what is called activation energy ok.

Now, here, but you we have at the same time we have another plot like this. So, here if you take a slope, this slope will give you the deactivation energy, Ed this is the this is the what is the energy required for the reactivation of the protein molecule that also we can calculate. So, both the activation energy and reactivation energy can be calculated when we study the effect of temperature on the enzymatic reaction.

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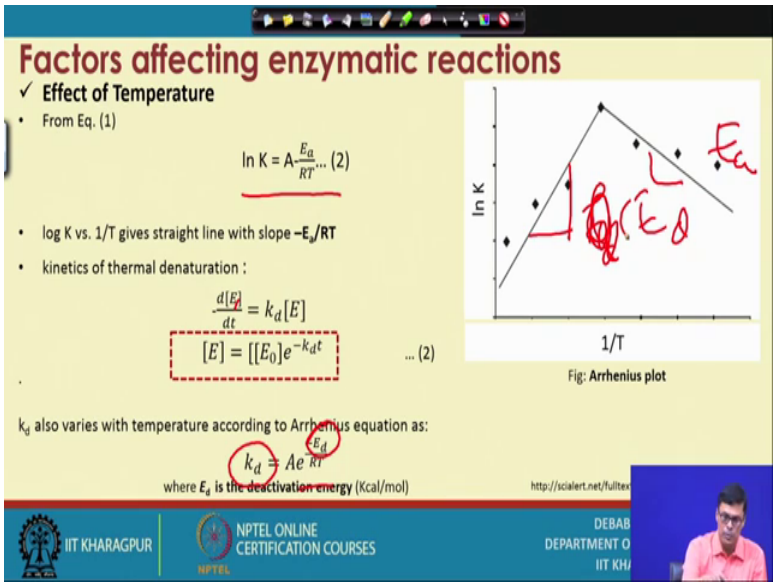

Now here we have we have shown the similar type of plots that as I mentioned before and. So, this is the equation that we have whether Arrhenius equation, and this is in this we can what this will help you to find out the k d value, that we can need the value and from that we can find out the E d value, this will this is a k d is that you know we if we consider d a rate of deactivation of the cell we can write d E by d t equal to k d into E.

Now, if you that this. So, this if we write k d equal to that this equation is there, and from that we can find out the value of E d. And here from this slope we can find out the E a and this plot we can find out I explained before also.

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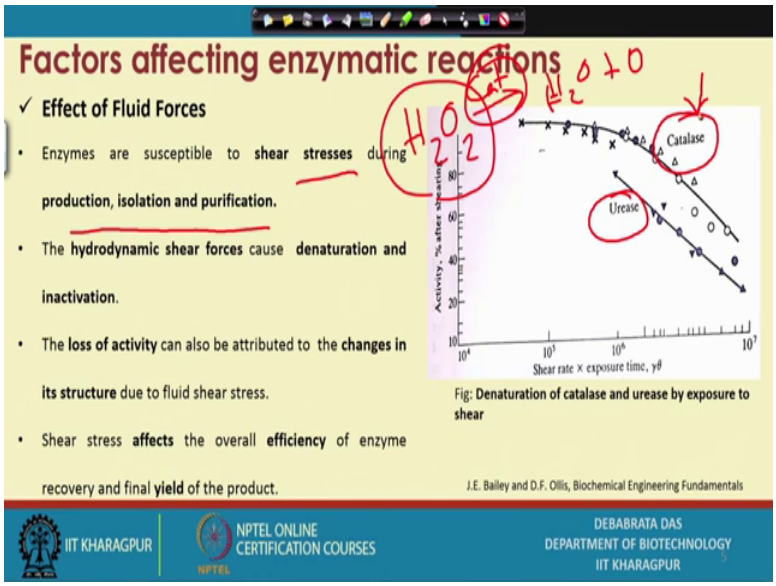

Now, next a very interesting thing is the fluid force let me show let me today I will show you how we can explain that.

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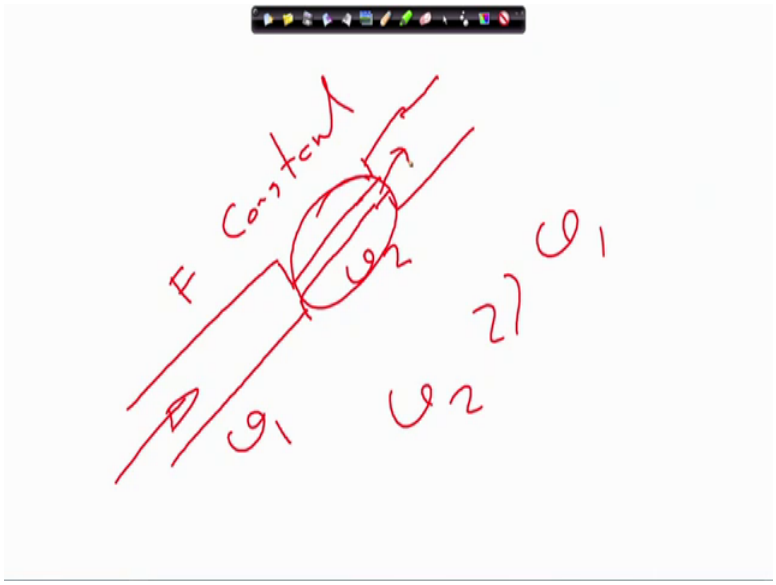

Now, suppose one liquid is passing through a pipeline like this, it is and all of a sudden you reduce the cross section area of the pipe. So, what will happen? The velocity that we have here v 1 and in this is v 2 now as soon as we reduce that if you have flow rate is constant flow rate is constant.

Then what will happen your v 2 will be much much higher than v 1 am I right. So, it will with the velocity increases drastically now as the velocity increases then there will be some kind of shear forces and due to shear forces it. So happens, your active site of the enzyme that will be denatured. But this denatured is reversible in nature what I want to mean, they are suppose again I put it in the higher diameter pipeline. So, as long as they are present in this narrow pipe, they are on the stress condition. They as soon as we release the stress here the some of the activity not 100 percent activity some of the activity of the enzyme they will get back to the system.

So, this is that is why I call that this is the reversible in nature, in case of the this is how shear force acts on the this. So, this is the enzymes. So, here you here you see the enzymes are susceptible to the shear stresses during the production isolation and purification. So, here I want to I have taken the example of catalase and urease enzyme; what is the role of catalase? We know all the H 2 O 2 that in presence of catalase it produces H 2 O plus oxygen am I right. And this present mostly this catalase enzyme present the aerobic organisms and obligatory ended up organisms they do not have catalase enzyme, that is why the oxygen is detrimental for their growth of the organism.

So, here this is it has been fine that this is what is called shear rate into exposure time. Now as the as this is increases, now it is like this though it is initially we do not have much of the deactivation of enzyme, but if you keep on increasing this exposure time then what will happen the activity will decreases. And in same thing happen in case of urease. So, what is the urease? Urease enzyme that acts on urea to liberate ammonia; so this decrease of the activity will take place, the hydrodynamic shear force causes the denaturation and inactivation activation the loss of activity can be attributed the changes in the structure due to fluid sphere. That is the exactly I am saying that is shear forces that will change the structure of the active site.

That is why when the substrate comes they will not get their proper sitting place at the active site; so your activity of the enzyme that will decrease. Now shear effect affects the efficiency of the enzyme recovery and final yield of the product. So, this is in addition through that another effect the physical forces that we have what we call surface tension. Now what is surface tension now when we know when you talk about any kind of biological fermentation process, then with the foam formation is the common characteristics of most of the fermentation process.

Now, due to formation of the foam that there always will be there will be interface between gas and liquid. And the in the in between the gas and liquid interface there were tremendous surface tension as dynes per centimeter that is a. So, usually we might be aware that the enzymes who is secret by the living cells there they usually having the sealed surface tension about one dined per centimeter, but here at 80 dines. So, naturally when they come in then interface that is every possibility, the enzyme will undergo the deactivation.

So, this is the how the different physical forces that affects the activity of the enzymes.

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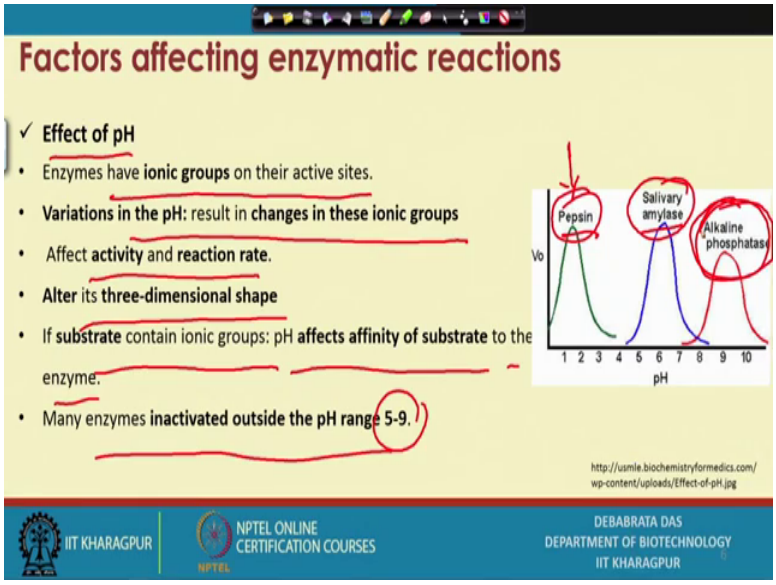

The next let me switch over to the effect of some kind of chemical parameters and the first that is most important thing is the effect of pH. The pH plays a very important role in the biochemical reaction and enzymatic reaction now question come why. The reason is that enzymes have the ionic group on their active site am I right their ionic group and variation of pH resulted them their ionic group like; what is the ionic group maybe it is positive, may be negative may be neutral, now suppose a active site we have positive.

Now, substrate is negative. So, what will happen? Your substrate will can freely interact with the active site and give the product. Now if the if the charge of the substrate and the active site is same, then they are surely repulsion and your due to repulsion the activity will be very less. So, that is why that is how this a pH a box, and effect of activity and there it affect the activity and rate of reaction, and altered the three dimensional shape if

the substrate contain ionic group, the pH affect the affinity of the substrate to the enzyme and many enzymes in activated outside this range 5 to 7 this is the range.

Now, here let me point out that that we know when we have come across different type of enzymes like I have given the example of pepsin salivary amylase and alkaline phosphatase. Now due to this different enzymes had different optimum pH for their maximum activity. And pepsin is kind of enzyme that present in our stomach am I right and we know our stomach pH is around 2. So, at two this is pepsin is kind of prototyping enzyme. So, this active as a pH 2 salivary enzyme amylase that is driven in our saliva and that activity is more or less close to the neutral pH that is now alkaline phosphatase usually that occurs at the at the at the alkaline.

This justify how that different enzymes are activated at different pH due to the different ionization characteristics of the enzymes.

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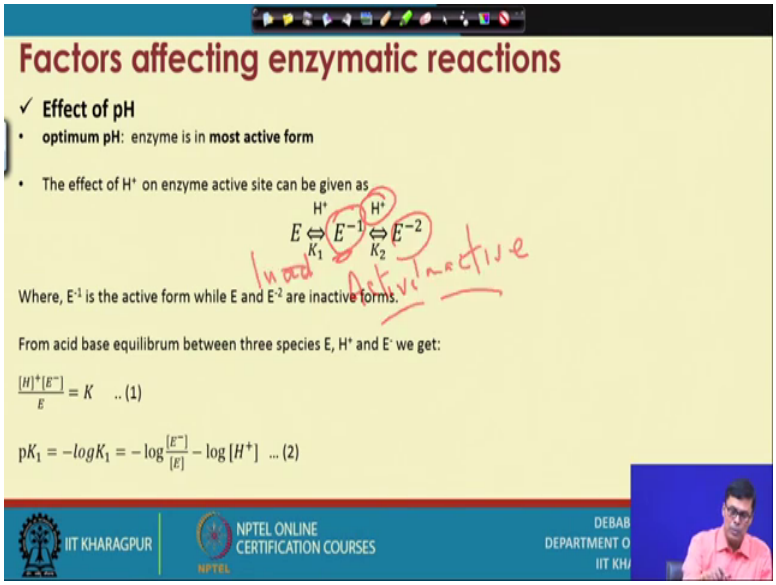

Now, this we want to go a little bit details on that that how the pH effect on the enzymatic reaction can be explained. Let us assume this is the enzymes you know which is inactive, this is in active am I right and this is also in active, now if this is let us assume this is the active. So, what is happening that when you that a the enzyme in turn that take out one hydrogen ion, then E minus 1 is there and this is we consider is active, but if you take further or one hydrogen ion then E minus 2 it is very becoming inactive.

So, you have the effect of hydrogen ion on the enzymatic activity can be explained very easily like this. Then when you remove one hydrogen ion then it is active, but when you remove another hydrogen ion it is become.

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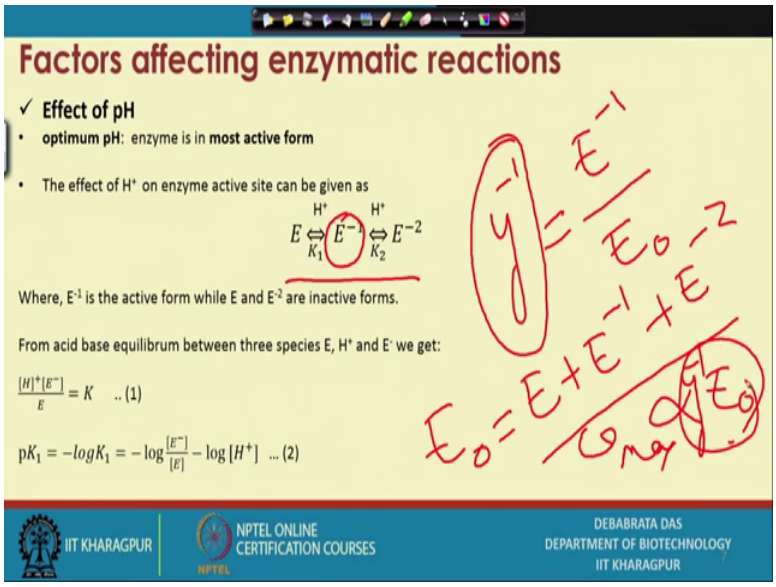

So, this is the optimum this is the thing now we have used one term called y minus 1.Y where is where is the fraction of the y 1 in E 0 what is the E 0? E 0 is there is the total enzyme concentration and how you can explain this E 0 in this particular, E 0 I can write this is equal to E plus E minus 1 plus E minus 2. This is the how total activity of the enzyme can be now we know that we have seen the velocity of reaction maximum velocity of reaction is proportional to E0.

Now, that a when we consider E 0 that means, that a whole enzyme that is active, but now when you use this kind of strategy then we find out only E minus 1 that is active; That means, y minus 1 this fraction of the E 0 that is active. So, this is. So, now, the v max I can write this is equal to y minus 1 into E 0. And then this is the enzyme that will proportional with the v max. This is how we can explain how we can express the how there are hydrogen ion, how we can affect the enzymatic activity now let us walk little bit in details.

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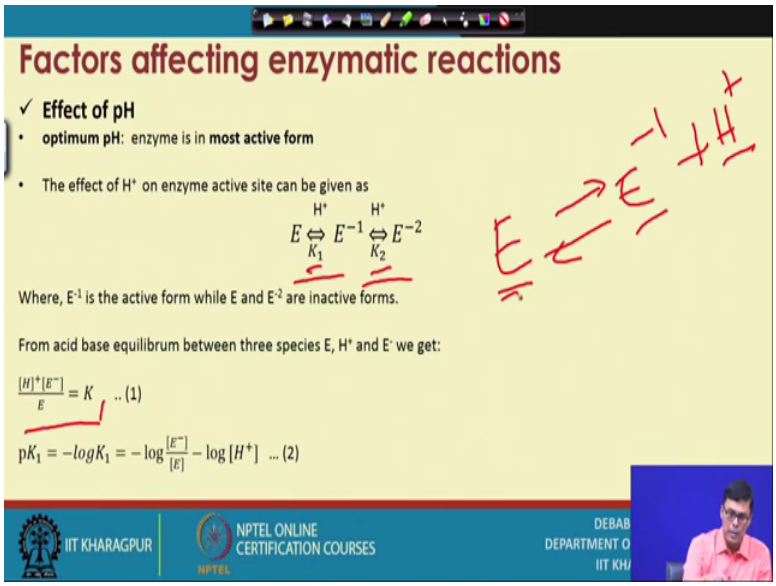

Now, when you consider the falls, let us assume this is reversible then this is also reversible. And equilibrium constant for this positive is K 1 and equilibrium constant for the second step is that is K 2. Now what is the K 1? This is equal to because this is like this E this is like this E minus 1 plus hydrogen ion concentration am I right. So, naturally k equilibrium constant this is K 1, I can write this is K 1 I think I we could not write it, that K 1 equal to hydrogen ion concentration with this product concentration and E minus 1 concentration divided by E concentration. So, we can write it very easily.

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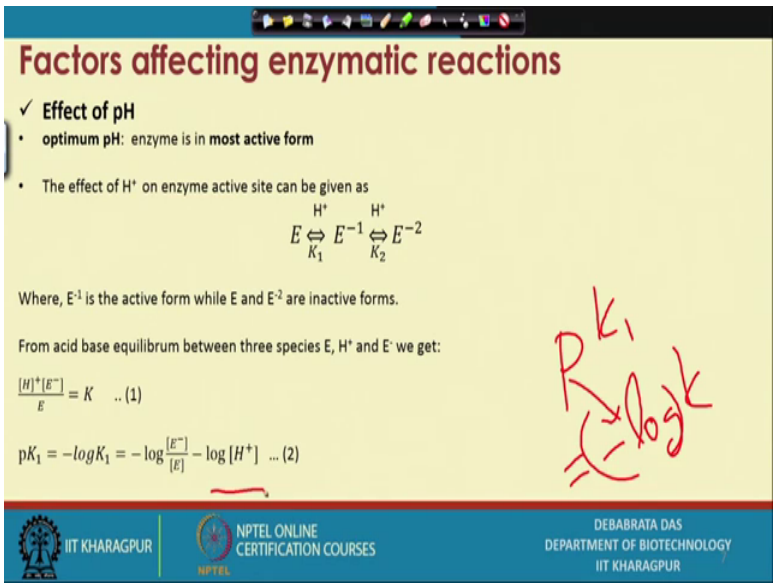

Then we can take log here what is P K 1? P p K 1 equal to minus log K 1 this is minus log p is transport minus log. So, this if we if we take the log we can we can write this like this that this we can write in this form.

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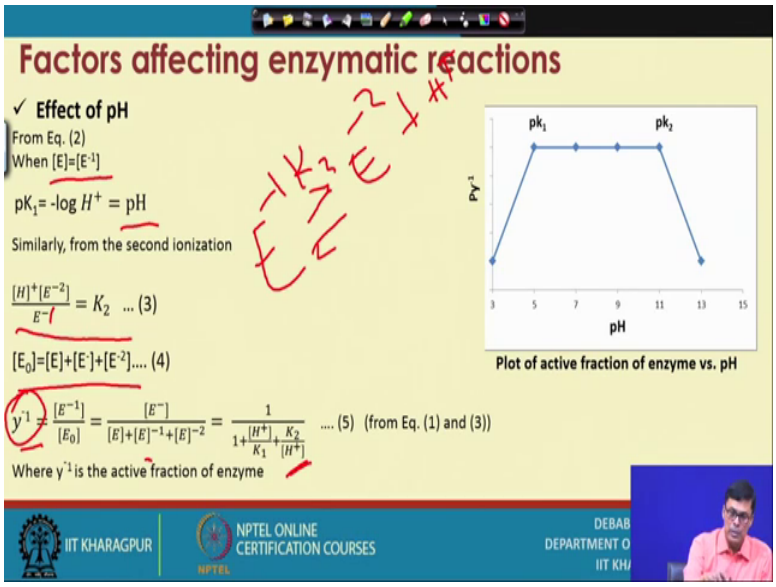

Now, next is that in the second step that we have. So, p K 1 equal to this again we can write p K 1 equal to now if you look at before that, now here we have seen p K 1 equal to this, this is like this. So, this is we have done that.

So, we can write that now we can write that p K 1 equal to minus log hydrogen ion, if we consider if we assume that E equal to E minus 1 if we assume that then that is log one that will log one equal to 0, and then we can write p K 1 equal to minus then this is equal to pH. And similarly in the second step that we have what is the second step we have? E minus 1 is plus minus this is equal to E minus 2 plus hydrogen ion am I right. So, we can write here that hydrogen and conserve E minus 2 is the E minus 1 this is one will be there equal to k 2.

So, this is k 2. So, this is equilibrium cosine E 0 I told you this is any point of time equal to u plus E minus 1 plus E minus 2. So, y then y minus 1 the fraction of enzyme that is that is the active enzyme that you can write E minus 1 divided by E, E plus this and this is equal to 1 plus hydrogen ion concentration divided by K 1 and K 2 by hydrogen ion concentration.

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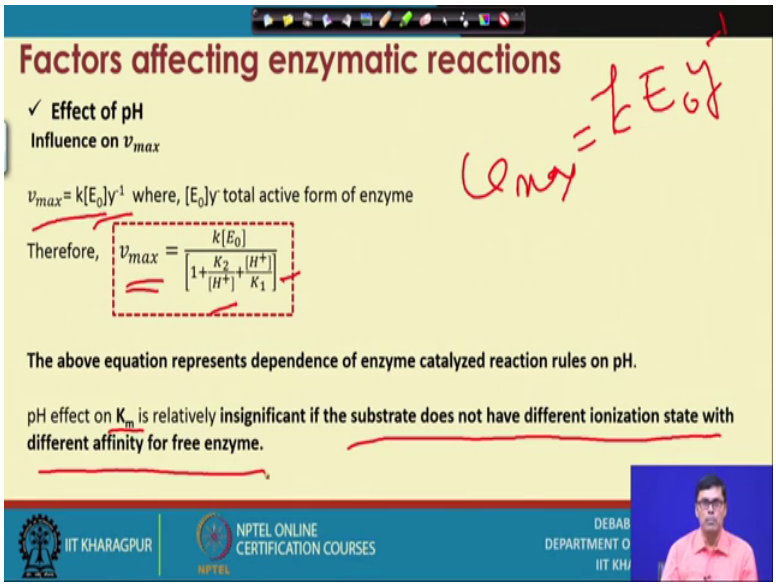

So, we can have a correlation between y minus 1 how it is affected with the with respect to the hydrogen ion concentration. Now this is the final equation that we have and then now here I forgot to explain that effect of pH on p y minus 1 this will as the pH changes we find in this range your enzymes are active this is the range that is active.

So, optimum pH will be in between this p K 1 to p k 2. And this is pH optimum is the hydrogen ion optimum method root over K 1 into K 2 and so, pH optimum will be equal to half into p K 1 by p K 2 this we can easily find out that. So, therefore, now finally, what we can write we can I told you that v max this already I explained you, that v max equal to I equal to what k into E 0 that we have done in before. Now E 0 all E 0 is not active. So, this will be multiplied by E minus 1 in the fraction factor.

So, this is exactly what we have written here and then we put the value of E y minus 1 here, then we can find a correlation how the v max depends on the hydrogen ion concentration. So, we can this is how a hydrogen ion affects the value of v max, but now question comes in the enzymatic reaction, there is a another constant called km the what does it km signifies? Signifies the affinity of enzymes it was the substrate or ordinal substrate affinity towards the enzyme. Now if the substrate does not have different ionization strain is the state with different affinity of the free enzyme, then we can assume the km is constant insignificant.

So, this is how we can explain the effect of pH on the enzymatic reaction. Now here we have given we summarize in this that how the different factors effects the enzymatic activities.

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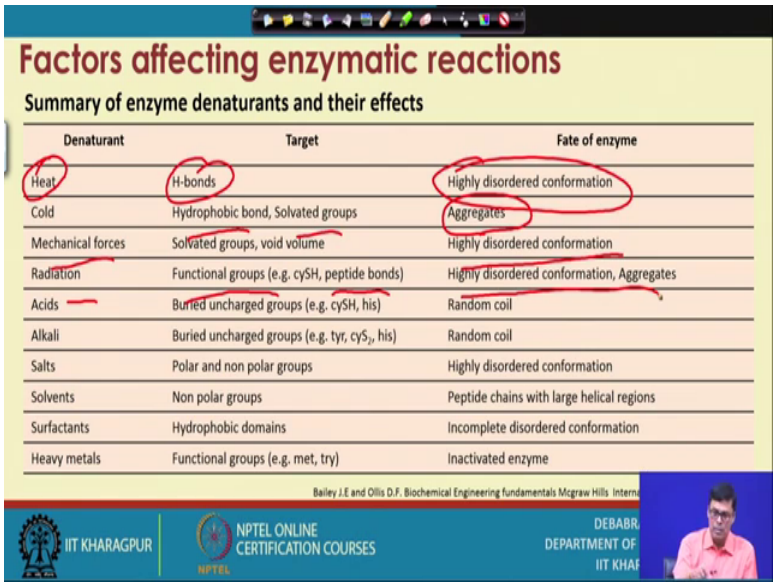

Now, here we have seen the heat, heat that effects the hydrogen bond and the highly disorder conformational changes that take place. Now cold also hydrophobic salvation and aggregation of the organisms that is the compound will take place mechanical force this is highly disorder confirmation. Radiation functional group like peptides and highly disordered conformation. So, this table give you some idea that how different-different factor different parameters influence the activity of the enzymes.

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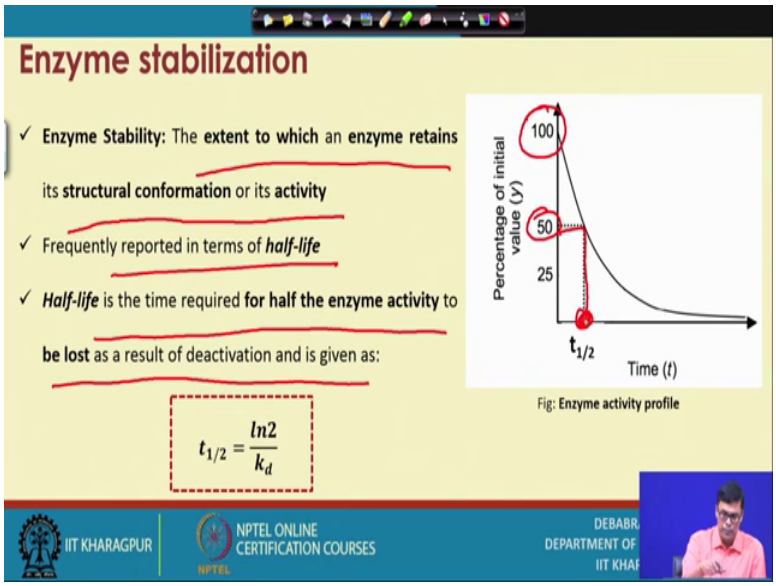

Now, first of all I want to discuss one very interesting topic what do you call enzyme stabilization. Now what do you mean by enzyme stabilization? The major drawback when we go for any kind of utilizing any kind of enzyme is that the stability of the enzyme why? Because then enzyme we cannot use for longer period of time, we know that in the day to day life we use the battery. The battery has two type of life one is called self life another we call operating life the same thing is the applicable to the enzyme also. Enzymes if we use for longer period of time automatically the activity will be lost and enzymes even you keep it in the fields for a longer period of time the activity of the loss.

Now, now question comes what parameters determine the enzyme stability, and the parameter that is find out the enzyme stability that is called half-life of the enzyme. Now what do you mean by half-life of the enzyme? The time required the half of the activity of the enzyme will be lost that is called half life of the enzyme. So, here we try to point out here the enzyme stability to extent with the enzyme retained the structural conformation and its activity, frequency depends on the half life and half life with the time required the half of the activity of the lost as a result of deactivation in the energy.

So, here suppose this is the 100 percent activity and this is the time. So, this is the 50 percent activity. So, here that whatever time is there, this we considered as they that half life of the enzyme. And this can be expressed a t 2 equal to l n 2 by k d. We have already

given you that a mathematical expression before that if you use that we can find out this equation.

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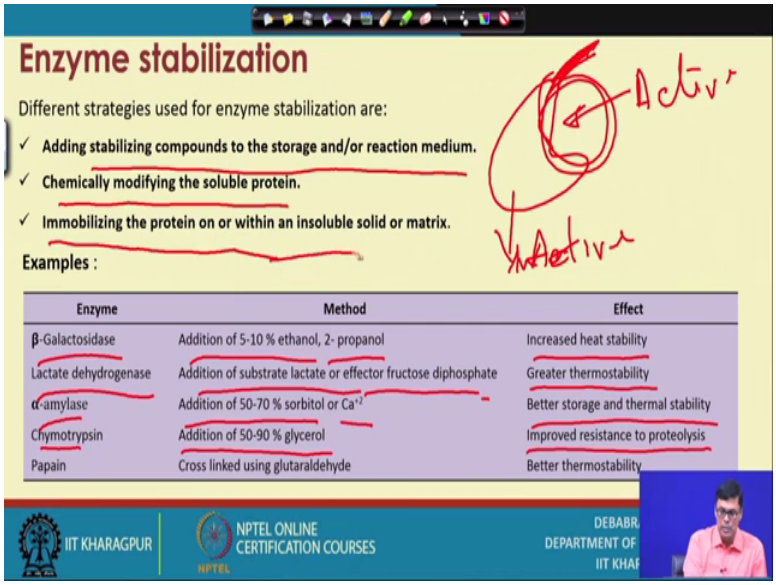

Now here the question comes what should be the strategy to increase the stability of the enzyme.

There are three different strategy there we have, one is by adding the stabilizing compound this is the adding stabilizing compound to the storage or reaction; example is the glucose that galactosidase. Addition of 5 to 10 percent ethanol and 2 propanol increase the heat stability lacto lactate dehydrogenase, the addition of substrate lactate or affected the fructose diphosphate greater thermostability; alpha amylase addition 50 percent of sorbitol 70 percent sorbitol or calcium ion the beta storage and thermal stability. Chymotrypsin addition of 50 to 90 percent glycerol improved the resistance to proteolysis. This is how different chemicals that increase the stability of the enzymes.

Another important thing is that the chemical and the modification of the soluble protein; because the enzymes we know that enzymes I told you the most of the part of the enzymes they say inactive. So, this is the inactive am I right this is the inactive, and this is active. So, this is very small fraction of the enzyme, now suppose the close to this it the poly some poly alanine group is there. So, there it has been observed that if you put some kind of poly alanine modified this active site it will make some kind of stability of

the active sites. Another way we can do that is the mobilization of the protein or within the insoluble solid matrix.

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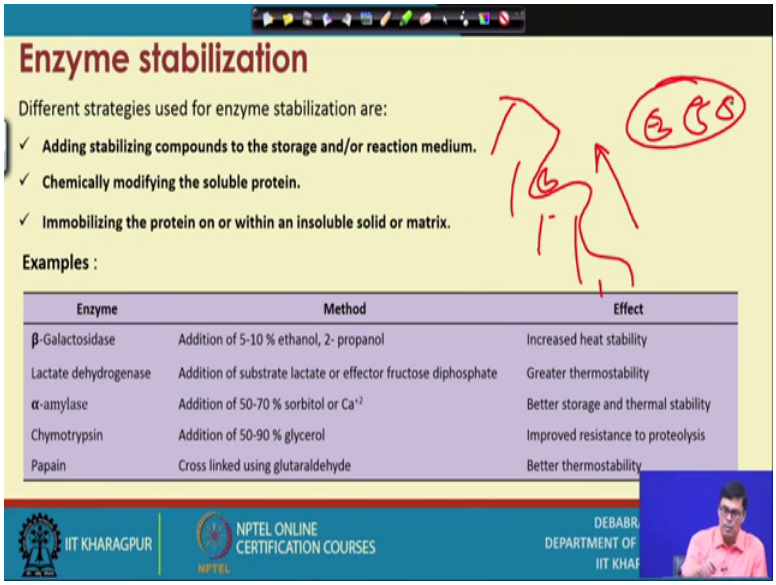

As for example, I can give you the example, suppose this is the solid matrix and this is solid matrix and inside the solid matrix if you have the enzyme. So, this is guy this is protected from outer this environment. So, suppose they immobilize you have put the enzymes in the some kind of envelope. So, it has some kind of protection inside this, and through which we can increase the stability of the enzyme.

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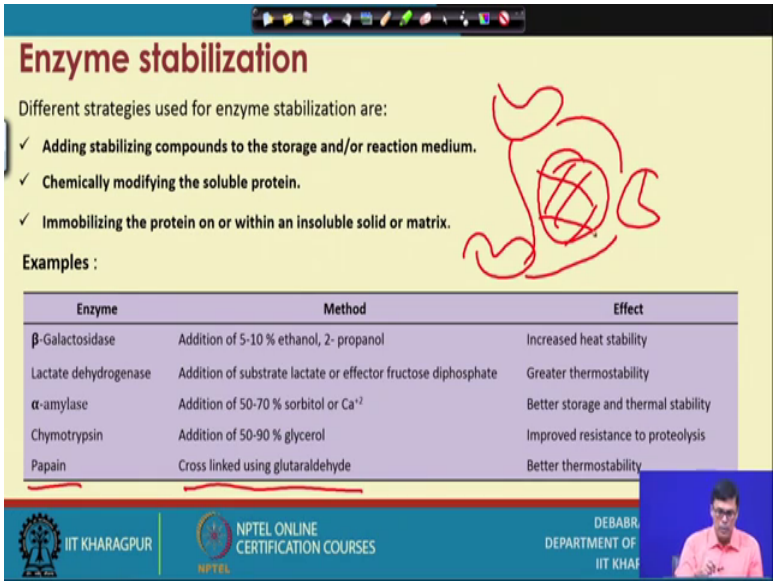

I can give another example is to here is a cross linking you know that is cross linking with pep popping that is available in the papaya how it increases activity by cross.

What is cross linking? Suppose this is the enzyme and we have this glutaraldehyde, this is the cross linking is not and this this binds here and this binds here and this is how they cross linked with each other. Now inside if you put some kind of solid matrix, this is embedded on the surface of the solid matrix and how the immobilization take place. So, this is the different way how can we how we can stabilize the enzyme.

So, in conclusion I want to tell that this particular lecture deals with that the effect of physical chemical parameters on the enzymatic activity, initially I try to explain how temperature fluid force and the surface tension effect the enzymatic activity, and then how the chemical chemically that activity of the enzyme can be affected with respect to pH, and we have seen we have determined the correlation between maximum velocity of reaction with respect to hydrogen and concentration. Also we find that km value is more or less it is remain constant if the ionization characteristics of the active site between the substrate enzymes remain same.

And finally, I shall discuss how the stability of the enzyme can be improved, and how you can express the stability of the enzyme, with the help of half life of the enzyme.

Thank you very much.