Aspects of Biochemical Engineering
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Lecture - 22
Kinetics of Enzyme Catalyzed Reactions Using Free Enzymes – II

Welcome back to my course aspects of biochemical engineering and the last lecture I try to concentrate on this kinetics of enzyme catalyzed reaction using the free enzymes. Then I try to explain that what do you mean by enzymes I told you that enzymes are basically protein molecules, but not necessarily their protein molecules some of due to the advancement of organic chemistry it is possible to produce some enzyme synthetically, but and then I explained that what is the nature of the enzyme, what is the nomenclature of the enzyme, then how a substrate and enzyme they interact with each other to give the product. Then I explained the how Michaelis Menten that proposed the rate of the enzymatic reaction with respect to substrate, then how Bricks and Hellen they pro they justify this Michaelis Menten equation with the help of reaction kinetics.

Now, later on I discussed that what are the limitations of this Michaelis Menten equation and what are the different significance of K m and V max paradigm. This lecture I am concentrating on inhibition of enzymatic reaction because inhibition is a very important aspects as for enzymatic reaction is concerned because it has several applications in the chemical and biochemical industries now I can give you a typical example that pesticide as you know it is a very complex molecule sign it very difficult to estimate in normal analytical techniques.

So, we shall have to use some kind of sophisticate techniques like h p l c and other things to find out the concentration of pesticides, now enzymatic reaction can be used inhibition we can use technique use to find out the concentration of the pesticide molecule another applications we have in the medical science I and the sulphur drug we use in the day to day life for curing some infection reduces. So, that also can be done I shall explained you how it can be done with the help of inhibition so.

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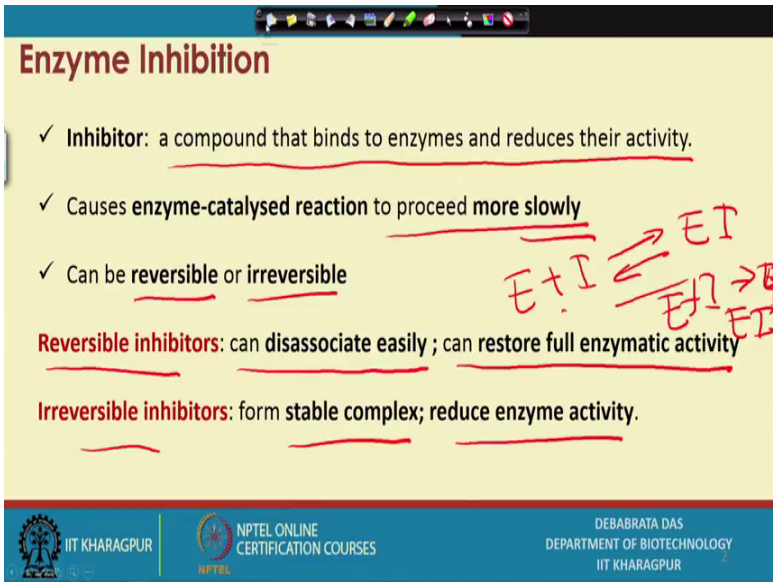

Let me start this lecture with this formation that what do you mean by inhibition, the inhibition inhibited a compound that binds the enzyme and reduce their activity. Now, what do you in a simple way what I can explain the inhibition mean retardation of velocity of reaction that causes the enzyme catalytic process slowly that inhibition does not means stopping of the velocity of reaction it means the inhibition rate of reaction is inhibited.

There are 2 type of inhibition one is reversible, another is irreversible, now what do you mean by reversible inhibition; that means, it dissociate easily that suppose E plus I is their inhibitor that reversible means this is E I and again it will go back that this is reversible and irreversible means EI this form E I this is this is like this E I that we have. Now, irreversible is stable complex and reduce the enzyme activity. So, one thing I want to highlight in case of reversible inhibitor it is possible to restore some of the enzyme activities.

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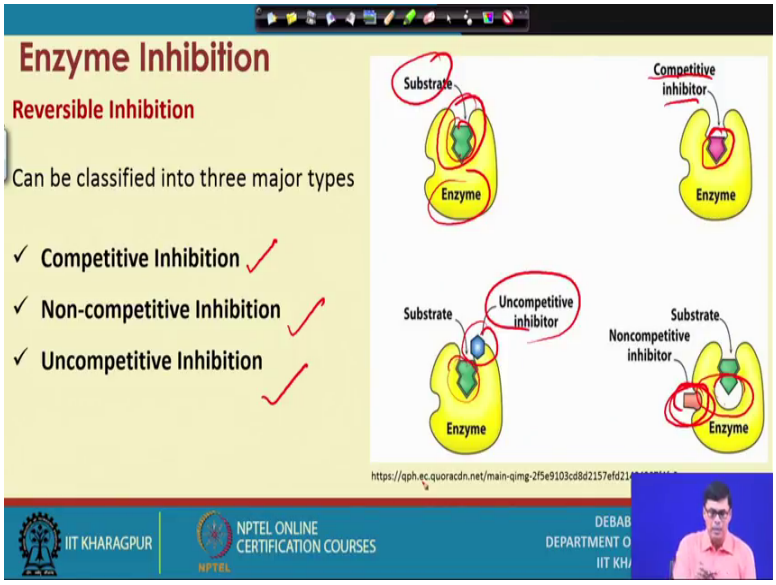

Now enzyme inhibition may be of 3 types the one is called competitive inhibition, noncompetitive and uncompetitive this is the pictorial representation of the inhibition the how this is the usually there normal case, this is the enzyme molecule and this is the substrate and this is the active site, now this is substrate should I say it at the active site and you can see the configuration here of the substrate and active site they are same and that is why they give the product.

Now, in case of competitive inhibition what is happening this inhibitor has similar configuration I with respect to substrate. So, it can compete that both the substrate and inhibitor they can compete for the active site, now in case of noncompetitive inhibition you see that they form the tri molecular complex this is inhibited this is substrate and due to the binding of the inhibitor the configuration of the active site changes that is why the substrate can you see it here properly and these since it cannot sit here properly, it cannot give the product and uncompetitive site this is the uncompetitive heat and binds here and you know substrate there finds here that they cannot give the product. So, these are the different 3 different inhibitor that we have.

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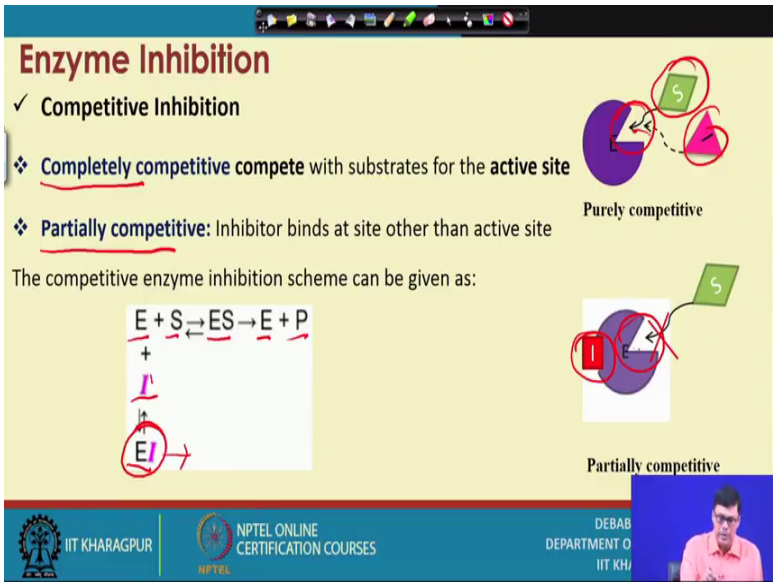

Now, let me go in details, what do you, how we can explain the this competitive inhibitor now if you look at competitive inhibitor that E is the enzyme am I right S is the substrate and for me E S complex and they did produce E plus P, but that I where E also react with I, I is the inhibitor and it from E I complex, when E I complex is there it will not give produce any product, but when E S complex is there it will produce product. Now competitive inhibition may be of 2 types, one is called completely competitive inhibition and the partially competitive inhibition.

Completely competitive inhibition means that when substrate and inhibitor they compete for the same active site and what is the partially competitive inhibitor that inhibited by else other than the active sites and then it will not allow the substrate to sit at the active site. So, it will change the configuration of the active site. So, that reaction will not take place. So, this is how we can define completely competitive inhibition and completely uncompetitive inhibition.

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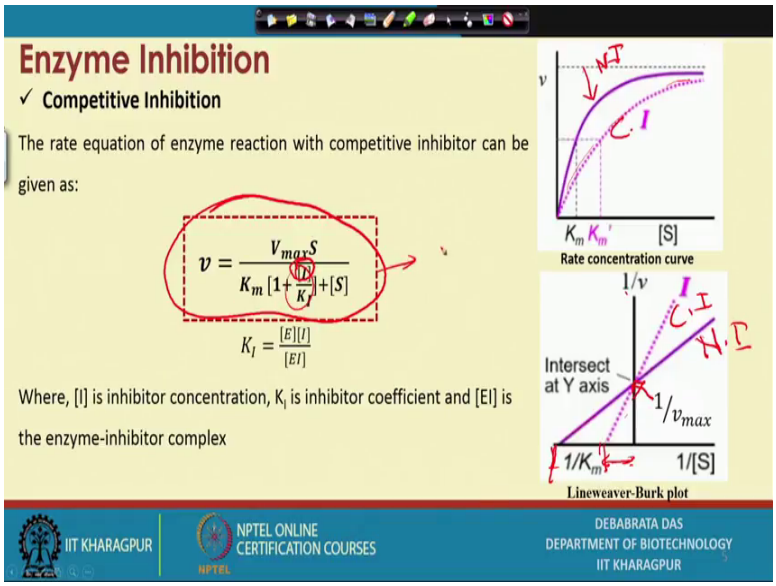

Now, how you can determine whether it is competitive. So, best way of determining if you plot V by S, V by S this is no inhibition am I right and this is competitive inhibition this is competitive inhibition this is like this. Now, if you this will be more clear if you plot Lineweaver Burk plot 1 by V versus 1 by S then this is no inhibition and this is competitive inhibition the competitive is like this.

Now we can remember I told you this intercept is 1 by V max the 1 by V max is remain constant, but this slope changes and since the slope changes what is this intercept with this intercept is 1 by K m and; that means, 1 due to inhibition 1 by K m decreases, 1 by K m decreases means K m value increases and if the K m value increases your rate of reaction decreases. now this if you analyze this equation that you know kinetics then we find that equation for the competitive inhibition is this V equal to V max S K m 1 plus I by K I plus S. Now, here I want to highlight if I put 0 then this will be 0, then this equation will be becoming the Michaelis Menten equation.

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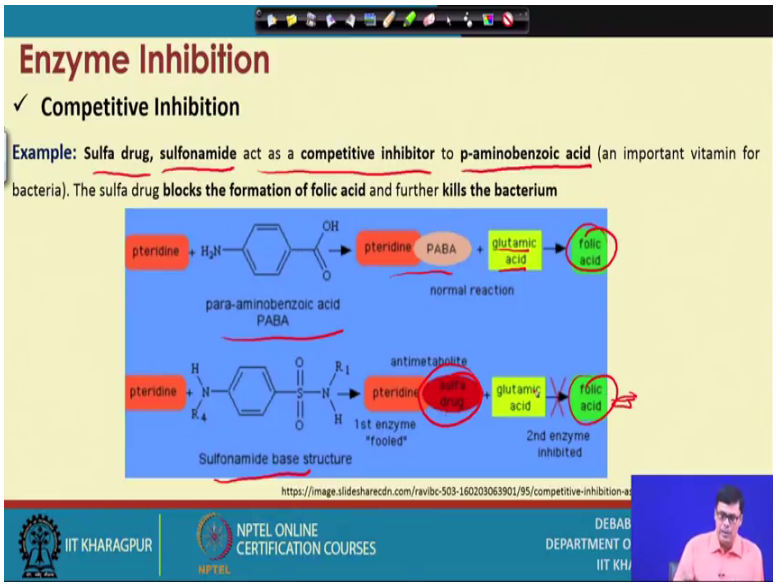

In the example site I was talking about Sulfa drug am I right, the sulfa drug is nothing, but sulfa amide acts as a competitive inhibitor with respect to this para-aminobenzoic acid now here you can see this is how that para-aminobenzoic acid they acts with this and then this form with the glutamic acid, is it form the folic acid and this folic acid is very much essential for the survival of the bacterial cells.

Now, in presence of the sulfa drug then what is that, this is the sulfa drug then this that enzyme molecules that binds with the sulfa drug and then it will not react with the glutamic acid to form folic acid since there is no formation of folic acid. So, your bacteria will be killed this is how sulfa drug works.

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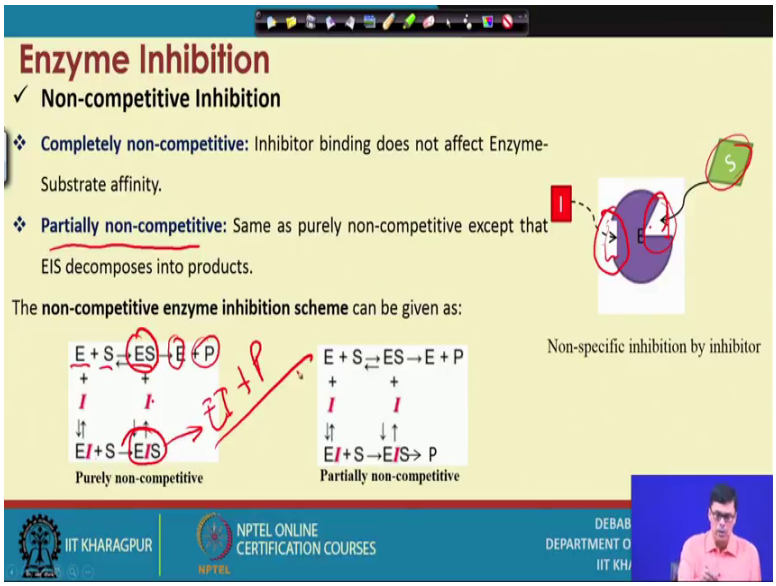

Now, next is the compete in noncompetitive inhibition this is the and in case of noncompetitive inhibition 1 important aspects is the tri molecular complex formation I can explain you E plus S equal to E S then I equal to E I here E plus I equal to E I I E S equal to E I S. So, this tri molecular complexes formation, but here is same as your competitive inhibition E S complex will give E plus product am I right. So, this is the difference that we have between the competitive and noncompetitive inhibition.

So, here if you look at pictorially that I that inhibitor binds the other than the active side then it change the configuration of the active size. So, that your substrate molecule cannot react, or you know it can sit here active site is this side, but it will not give any kind of product molecule. So, inhibitor can binds here and substrate can bind here you do not give any kind of like this. Now they here also there is a term called partially noncompetitive inhibition what do you mean by that, partially noncompetitive inhibition means in normal case the E S will be produced the enzyme and product am I right, but in case of partial competitive inhibition product formation also will take place from the triple tri molecular complex then we call it partially noncompetitive inhibition.

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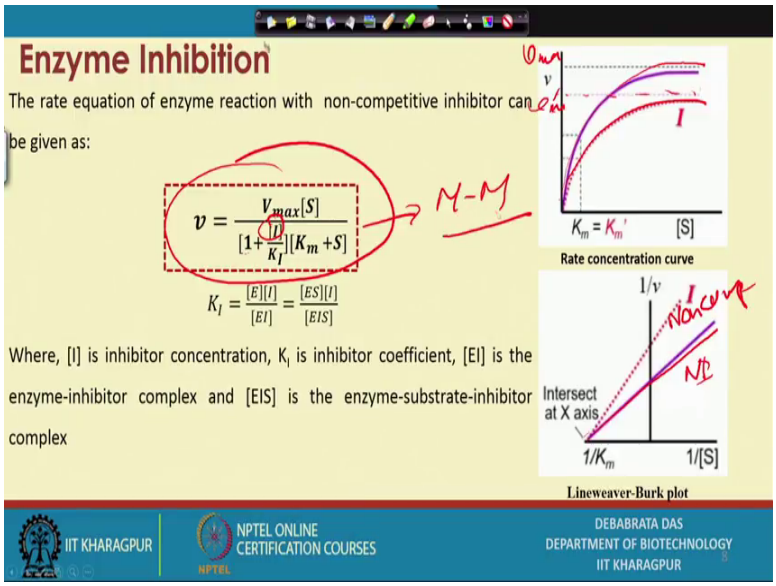

Now, the if you plot the Michael that you know that V by V by S plot then it will nature is like this is no inhibition this is noncompetitive inhibition. So, here we may I K we can see it V max value is changing this is V max dash and this is V max. Now if you plot the Lineweaver Burk plot then the plot will be like this is the in case of no inhibition this is noncompetitive inhibition. So, we can with this equation is coming like this v equal to V max S 1 plus I by K I K m plus S now here also if you put I equal to 0 and then this will be 1 and then it will be approaching towards Michaelis Menten equation.

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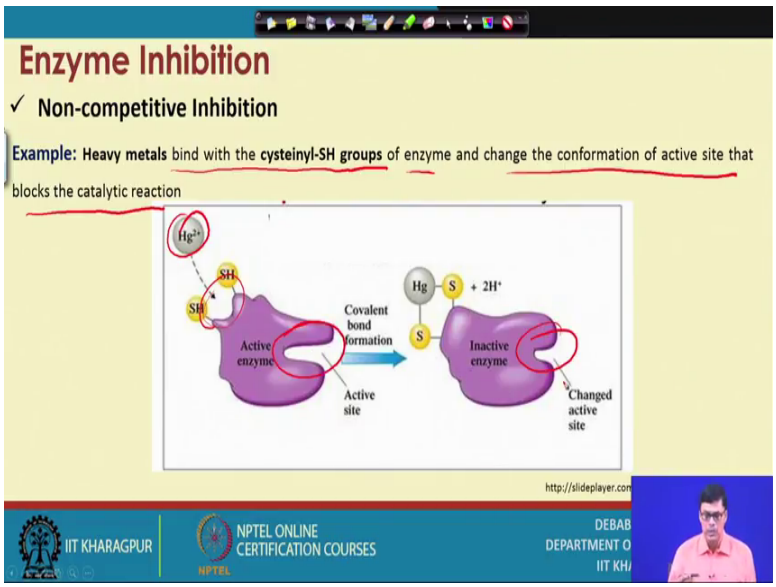

Now, this is the how it can be pictorially represented the heavy metal binds with the enzymes Cysteinyl S H group of the enzyme and change the contrary the conformation of the active site that blocked the catalytic activity, now if this there is mercury molecules when bind this side then you can see the how active site changes this is how your enzymes will be inactivated.

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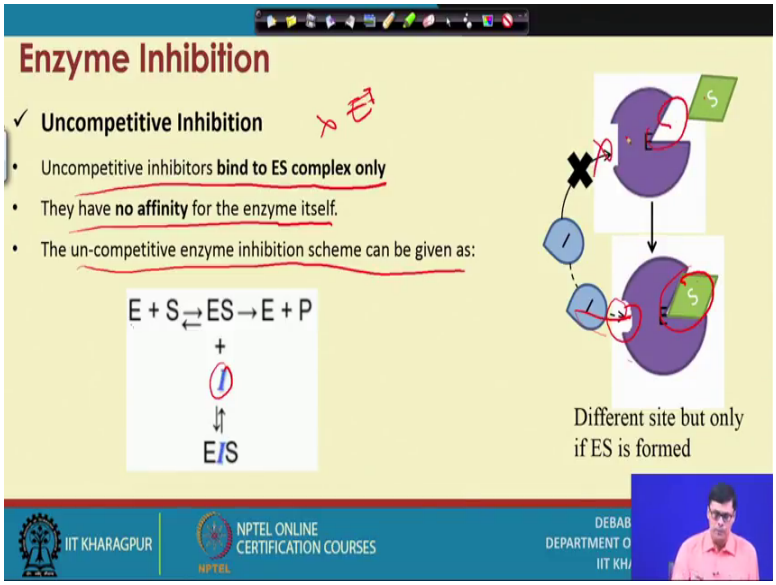

Now there is the another inhibition called uncompetitive inhibition what do you mean by uncompetitive inhibition that that E S complex binds with the inhibitor and from E I S complex, but here that E S that E cannot binds with I from E I complex and E I can cannot find the dye tri molecular complex.

The uncompetitive inhibitors binds with the E S complex only not with the E I complex am I right that this cannot be bind there is no inhibit that the affinity for the enzyme itself the uncompetitive enzyme inhibition scheme can be represented like this is, I is the inhibitor and substrate and this cannot bind here, I individually that I cannot bind, when substrate bind with the enzyme then and only then inhibited bind, when substrate does not bind here this cannot be binding.

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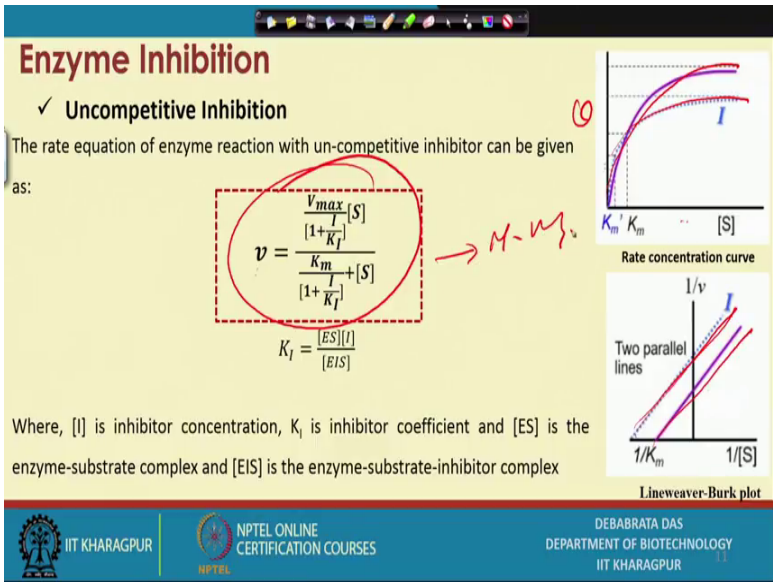

The nature of the plot Lineweaver Burk plot is like this that nature of this is V if you look at this is V versus S plot and we this is no inhibition, but this is the uncompetitive inhibition now if you look at here this is the noncompetitive inhibition this is no inhibition. So, here what is happening that V max and K m value both it decreases and in case of uncompetitive inhibition this equation also this case also V can be expressed like this here also if we put I equal to 0 then V will be becoming Michaelis Menten equation.

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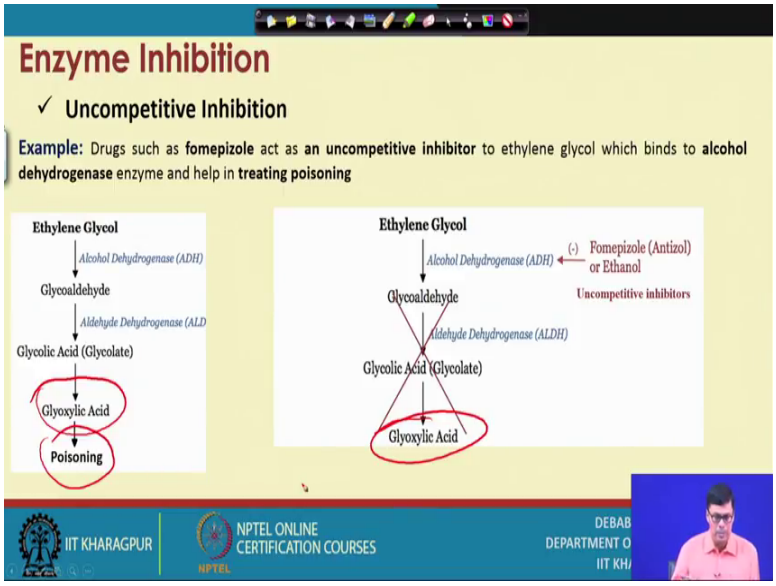

Now, this is the example that we have that Ethylene Glycol in presence of alcohol dehydrogenase and subsequent days we produce the Glyoxylic acid this acts as a poisoning effect, but when this Fomepizole that is Antizols are present they or ethanol presence then this binds that this uncompetitively and this will not produce any kind of Glyoxylic acid. So, this is how the bad effect they can be removed

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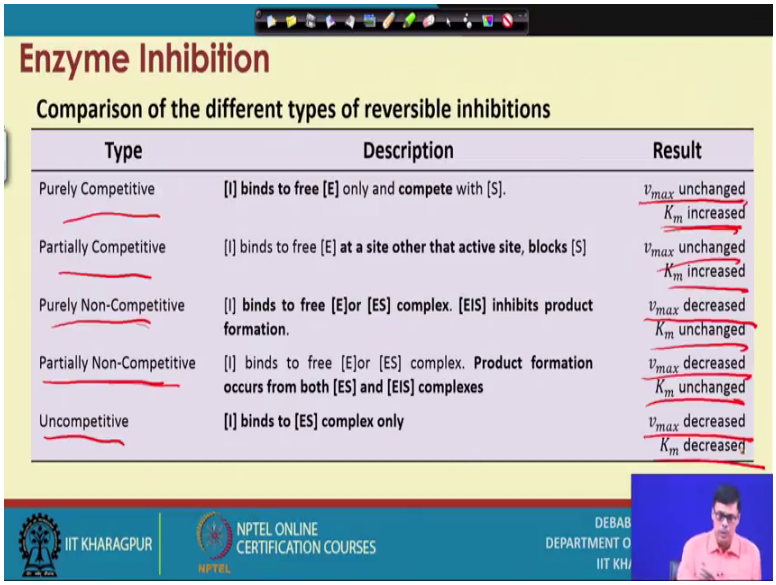

Now this is summarized in this particular table that this is purely competitive inhibition and partially competitive inhibition both the cases the same thing V max unchanged K m increases, but in case of partially noncompetitive inhibition a purely complete noncompetitive inhibition and partially noncompetitive inhibition V max the decreases K m unchanged here also V max it decreases K m unchanged, but in case of uncompetitive inhibition V max the decreases K m also decreases this you should remember.

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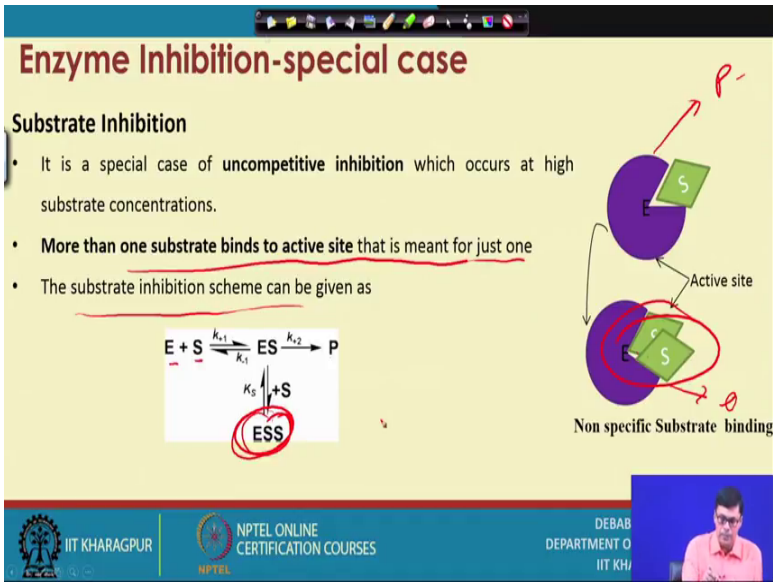

Now, there is a another interesting things there is substrate inhibition during the enzymatic reaction that this is how it is take place when there is the excess of substrate the excess of substrate binds with the active site what I was showing here when more substrate is they accumulated the active site no product formation will be there only one substrate is bind this will give the product, this is like this the E plus S E S and this will give the P plus S and when you have the E S it would not give any kind of product more than 1 substrate bind with the active site that is meant just to have 1 and the substrate inhibition scheme is represented like this and this is kind of example of uncompetitive inhibition.

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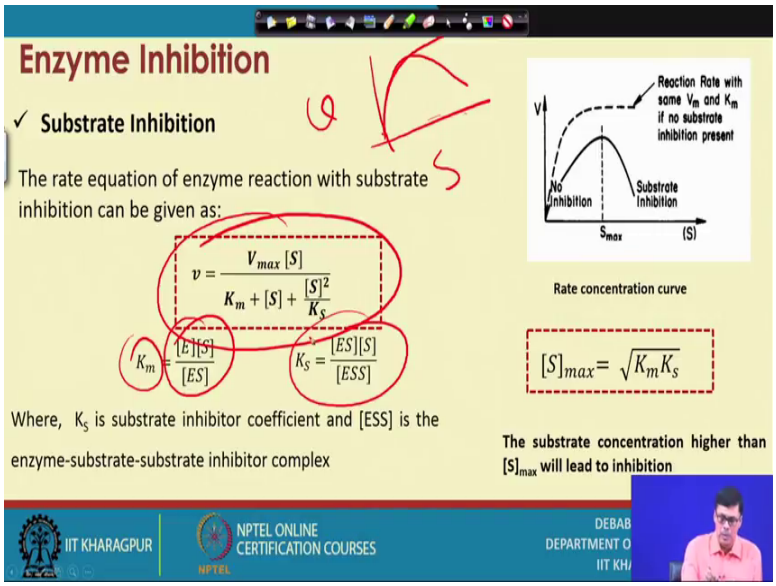

Now, this is this is the actually the case in case of normal V by S we have this is the no inhibition, but in case of substrate inhibition this will be like this. So, you are very after some. So, when we keep on increasing the substrate concentration the velocity of the reaction decreases and this axon that expressed by V equal to V max S K m is plus S square by K S this is this reaction can be explained and K m value is the E S by E S complex E S plus S equal to E S S complex that we have shown similar to the uncompetitive inhibition.

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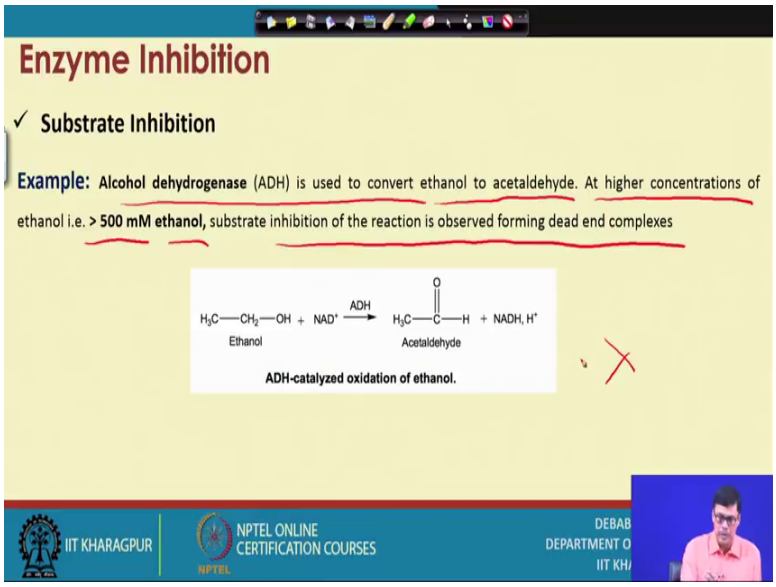

Now, this is the example that I have given you that is in case of Alcohol dehydrogenase is used to convert ethanol to acetaldehyde at a higher concentration more than 500 milli moles of ethanol substrate inhibit the reaction is observed forming the dead end complex. So, this is the normal reaction that we have, but more substrate is there then this catalytic reaction would not take place.

Now there is a there is a term called irreversible inhibition.

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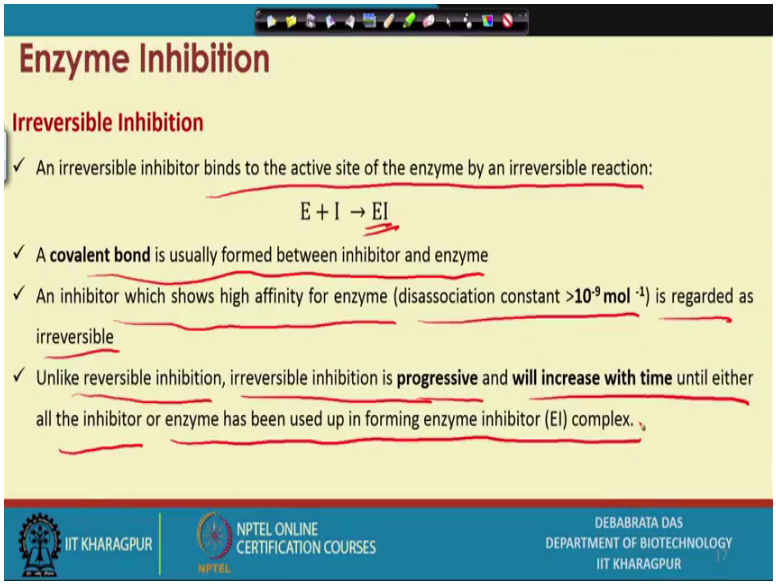

In irreversible inhibition binds with the active sites of the enzyme and the after formation of this E I complex it will not go back and covalent bond usually form between the inhibitor and enzymes an inhibitor which shows the high affinity of for enzyme disassociation constant is greater than 10 to the power minus 8 moles inverse is regarded as irreversible, unlike reversible inhibitor irreversible inhibition progressive and will increase with time until either the in the all inhibitor or enzymes are used of forming the enzyme inhibitor complex.

So, all inhibitor until unless the irreversibility they bind with the enzyme then the inhibition will keep on going on and then when all enzymes they bind with the substrate then and the inhibition effect will not be work that you know in this system that is the that is what do you call irreversible with the inhibition.

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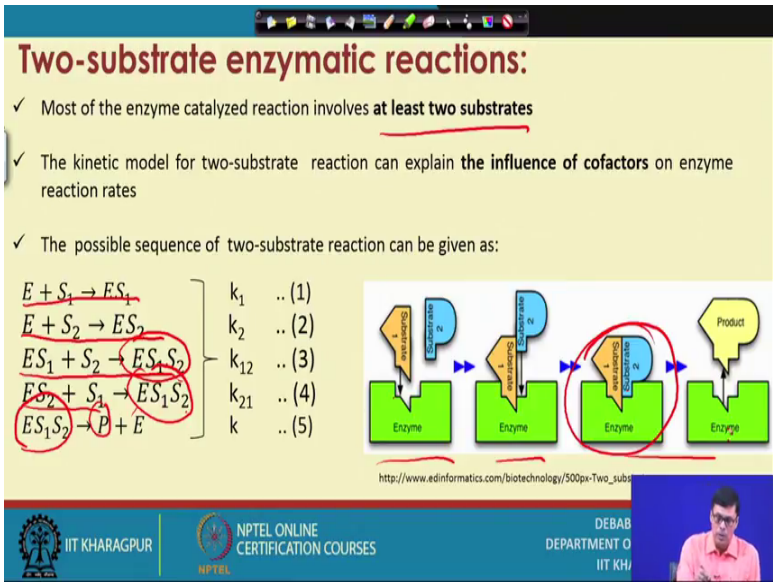

Now I told you when I discuss the Michaelis Menten equation that one important here that limitation is that Michaelis Menten equation discuss 1 substrate and 1 enzyme, but you know it does not discuss that 2 substrate 1 enzyme. Now, if you look at that most of the biochemical reaction particularly in the metabolic pathways you will find most of the reaction is taking place in presence of the cofactors and since most of the in presence of the cofactor initially by in the last lecture I showed you apoenzyme plus cofactor is a holoenzyme that mean in presence of the cofactor only the enzyme will be activated.

So, normal case that it is an example of enzyme catalytic reaction is an example of a 2 substrate and 1 enzyme now question can how we can explain, this is the possible scheme that we I try to explain that you know how to substrate mechanism can be explained E plus S 1 it provides the E S 1 E S plus S 2 E S 2 E S 1 plus E 1 E S 1 plus S 1 this from the tri molecular complex E S 2 plus S 1 tri molecular E E S 1 S 2 and whatever E S 1 is there that gives the product and enzyme and this is how it pictorially it we can explain like this that you know 1 substrate sit here then the second substrate sit here and when both the substrate sit here they gives the product this is what happens in case of coenzyme .

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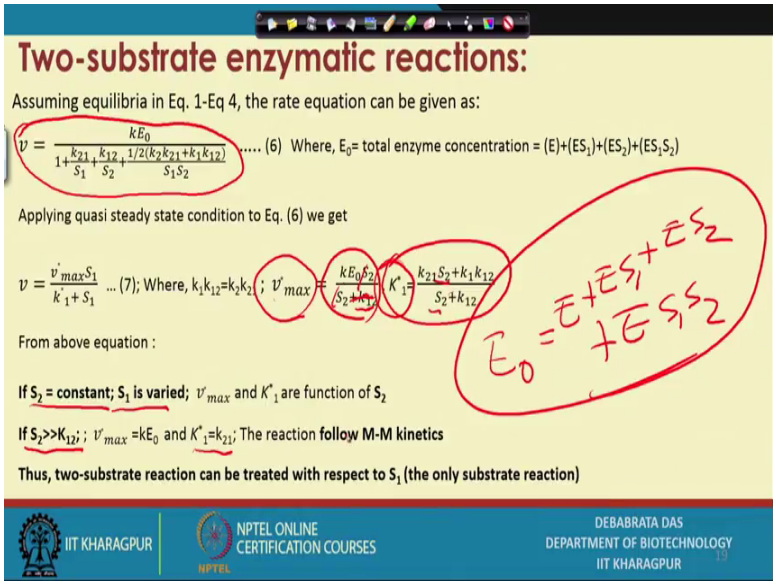

Now, when we analyze this particular reaction scheme we will come back this kind of derivation that the K equal to K is the rate constant S 0 is the initial enzyme concentration. Now here E 0 will be what E 0 equal to E plus E S 1 plus E S 2 plus E S 1 plus S 2. So, this is the combination of all this thing we call considered as the E 0. So, this is called bound enzyme, this is called free enzyme and then this equation will be becoming the Michaelis Menten equation if we assume that the V max star equal to E K into E 0 is S 2 and S 2 plus K 1 and K K 1 star equal to this.

Now, in this equation if you would see that the S 2 term is there and if you put S 2 is constant and S 1 is varied then what will happen if S 2 is constant, K is constant, S 0 is constant and K 1 K 1 2 is constant. So, I can assume V max V max star is constant now.

Here also if S 2 constant and then everything is constant. So, I can assume K 1 star is constant am I right. So, now in the second case if we assume S 2 is very large as compared to a K 1 1 2 and then what is happening that they very large then I can ignore this if we I ignore this then this will cancel then V max equal to K into S 0 that is the constant.

Now, K 1 also is they will be becoming this I can ignore as compared to this S will can cancel S 2 S 2 will cancel and K 1 star will be equal to K 2 1. So, this is the 2 substrate a model will approach towards the single substrate model when we assume 2 different cases, 1 case here do you assume S 2 is constant and another is the case the S 2 is much higher as compared to S 1 .

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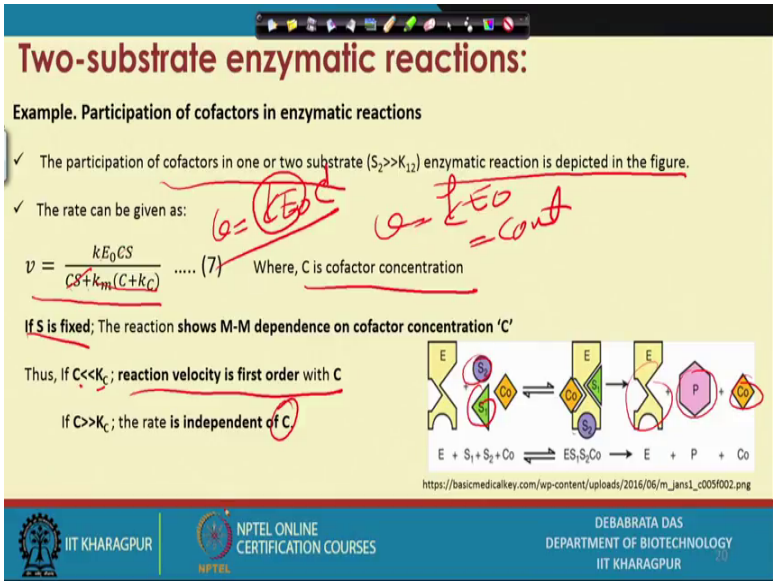

Now, let me discuss the coenzyme that how participate in the reaction that reaction kinetics how can be explain now the this the participation of the cofactor in is a 1 or 2 substrate this is the enzymatic as depicted in the picture that you see that this is the substrate and this is the S 2 is the substrate this is a cofactor and then this is cofactor binds here it makes the change of the active site. So, that we will get the product and the coenzyme and the enzymes that we have, now this we can express as that V equal to K into E 0 and C S C S plus K m plus C into K C, K C is the cofactor concentration.

Now, if S is fixed or S is constant and if a K is much smaller as compared to K C then it is much smaller than I can ignore that. So, then what will happening that it will follow

the first order kinetics this is will the K into then V will be equal to equal to K into E 0 into C. So, since it is constant, we can follow the first order kinetics with respect to C. Now if C is very large then I can ignore this is then what is happening that this C S I can ignore that then this C S will cancel then V will be tends to V max that you know the K K into E 0 V will be what K into E 0.

Now, it is; that means, it is equal to constant; that means, it is independent of C if C is very high then we can assume this is independent of C. So, in this particular presentation I try to discuss the inhibition aspects I told you inhibition is a very important aspects as well as for enzyme catalyzed reaction take place because it has a lot of application in the chemical and biochemical industry particularly for analytical purpose I shall show you some problem then how we can solve the how we can determine the concentration of pesticides present in a sample and this I showed you how this can be used as a drug, but I have given the example of sulfa drug there different type of inhibition that we have been reversible inhibition some of the activity we can get back.

But irreversible inhibition we lost the total activity of the enzyme then whatever inhibition is that probably it can be classified into 3 types 1 is competitive inhibition, noncompetitive inhibition, uncompetitive inhibition. Now in case of noncompetitive inhibition with the V max remain constant and K m increases noncompetitive inhibition V max decreases and K m remain constant, but in case of uncompetitive inhibition both V max and K m value decreases and then I try to explain how the 2 substrate model can be explained because the major limitations of the Michaelis Menten equation is this it is a single substrate model and 2 substrate model the way I try to discuss and I also discussed under what circumstances 2 substrate model stains to the single substrate model and also I discussed how the coenzyme reactions in the enzymatic reaction can be expressed.

Thank you very much.