**Biochemical Engineering**

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**Module No. # 01**

**Lecture No. # 12**

**Effects of Substrate and Inhibition, pH and Temperature on Enzyme Activity**

Welcome back to this 5th lecture in the series on Biochemical Engineering and this is on inhibition, we are continuing on inhibition which is substrate inhibition.

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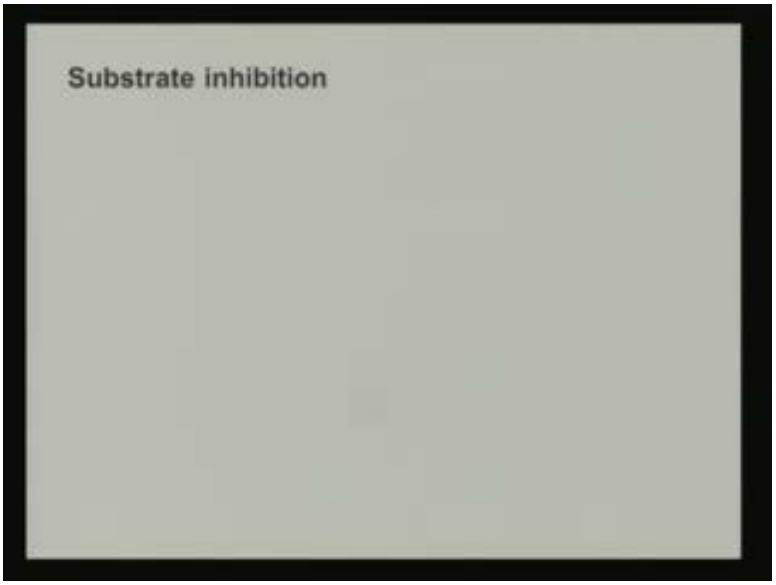


But, what we will do is we will pick up from you know, before we go into the details of substrate inhibition, we will pick up from where we left. And one of the things that, we are discussing in the last class was the question of multiplicity, and the fact that there could be, if you have auto catalytic reactions there could be a reactor run away.

So, why I am doing this and this is connected to both the substrate inhibition and what we are going to study in today's lecture, where does this multiplicity come in from one

of the things that, we have to try and understand is where does this multiplicity come in from And one of the answers I gave or you know comments I made in the previous class is that, it has to do with the, mathematically speaking it has to do with the, what the algebraic.

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Yeah certain non-linear form of the equation not all non-linearity will give rise to multiplicity. So, if you are dealing with a reaction kinetics, a reaction kinetics that can that has quadratics or some other form of non-linearity, that leads to more than one solution, so as we know that most non-linear equations lead to more than one solution right, but are all those forms, of interest to us or all the forms that lead to multiple solutions are are interest to us, when we did dealing with reactors, probably not, the reason being that, we are only interested in forms that lead to feasible solution.

So, if your concentration for example, is outside the feasible range, if it is negative or if it is a number that is much higher for example, your initial concentration say is 10 micro molar and your remaining concentration after 15 minutes or something or if you get it to be 20 micro micro molar obviously that does not make any sense. So, it has to be within the feasible range of operation and so if we have multiple solutions within that feasible range of operation, then we can have certain kind of multiple, multiplicity behavior.

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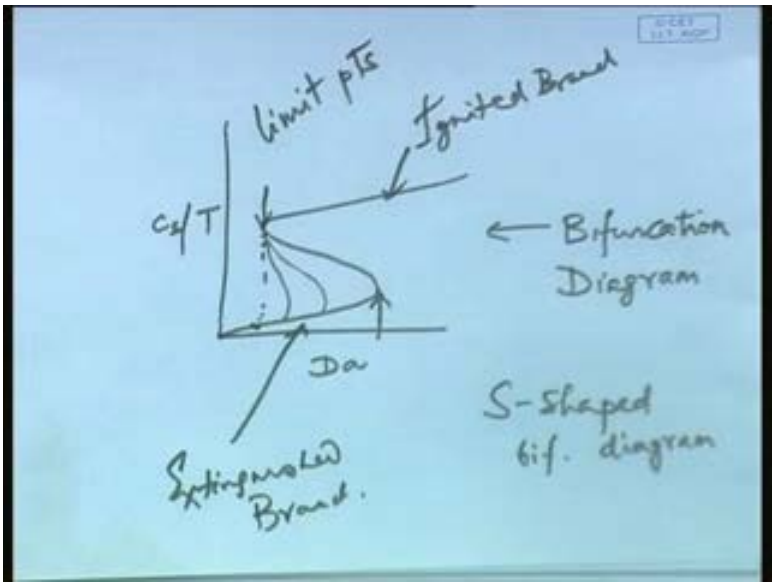

So, you know one of the things that, I want to show today is this, these plots and that you see on screen now, so this is, if this is say concentration and this is known as typically known as dam colon number, which is measure of the reactor resonance time, this could be concentration or this could be temperature; so when you have multiplicity in the system and we will do this later in the course a little bit more, so you get a plot this type right, this is called bifurcation diagram. So, in the bifurcation diagram and these two points, this is an S shaped bifurcation diagram, and would any of you have an idea what these two points are called?

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Yeah but, that is that is different from a reaction engineering point of view, from a mathematical point of view what are these two points called? They are called that that is right, one is an ignition point and one is the extension point.

So, which one is the ignition point?

(())

This one

Right one

This

(())

No but, this you are igniting it here right and you are extinguishing here, so you are igniting the solution here.

But, will go from starting from here

No but, yeah but, the thing is

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Thing is

Sir, rightly (())

Rightly to ignition yeah, so see the thing is that you may not always trace this path between these points, several paths are possible, this could be one of the path, this could be one of the path, this could be one of the path, you see what I am saying, so between these two points several paths are possible (Refer Slide Time: 04:23).

So, this is not the unique path between these two points, but what is important is that here, you are ignited you are extinguished, the solution is extinguished that is you have the low temperature or the low concentration, and then its suddenly goes to the ignition point, that is it ignites, the top branch is a ignited branch ignited branch and the bottom branch is the extinguished branch, so branch. So the point of ignition is a point where it where the top branch it starts fine, now what I wanted to know is mathematically would you know, what these two points are called, this point and this point (Refer Slide Time: 05:12).

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No, these are called limit points these are called limit points, so this is the simplest form of bifurcation we have, so bifurcation is essentially means multiple solutions at one particular value of the concentration.

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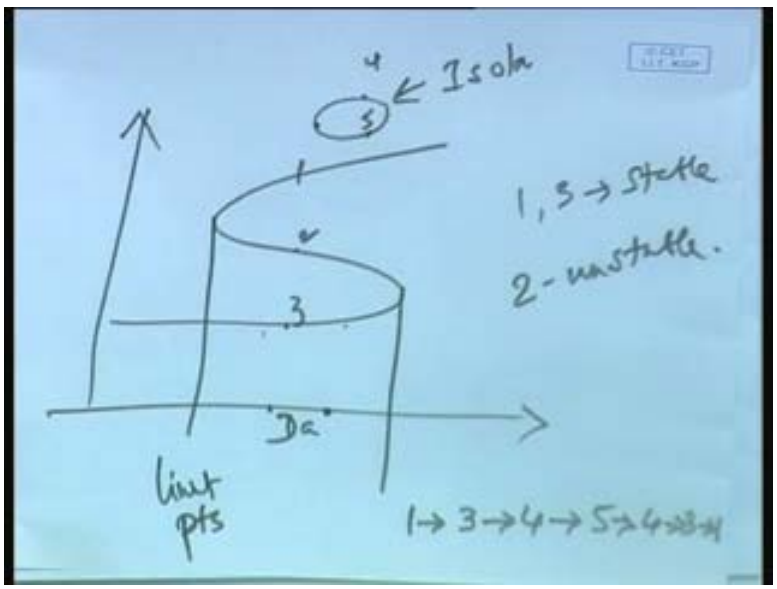


So, what I mean by multiple solution is that, so at this within the limit point, so this is one limit point and this is the one limit point. So, at a particular value of a dam colon

number you have 1, 2 and 3 solutions 1, 2 and 3 solutions fine, what we will examine later in this class is some of the questions as stability and so on and I have just to the prelude I can tell you that, these solution 1 or branch 1 and 3 are stable and branch 2 is unstable fine; so just as a prelude, but why these are stable and that is why these these two are stable and the other one is unstable, we will do later. But, this is the simplest form of bifurcation, then you can have other kinds of bifurcation, you can have pitch 4, you can have you know these island like structures, so if you have if you have an, this is called an isola, so this is an isolated branch, this is called an Isola.

So, when you have an Isola at this particular dam colon number, how many solutions you got? 3, at this particular dam colon number 5, so 1, 2, 3 these three branches are there, so Isola is there along with the by, it should be bifurcation diagram 1, 2, 3 and then 4 and 5, so 5 at this point for example, you have 4, the 1 for the tangent to the isola and then the and here again 4 and then here again 3 and then 1, so you have, you go from 1 solution to 3 solution to 4 solution to 5 solution to 4 solution to 3 solution to 1 solution, that is how we proceed? So, this is just you I need you to understand this concept, because in standard classical chemical engineering, you are not used to the whole idea, so when we are talking of you know this as a mathematical concept that is one thing.

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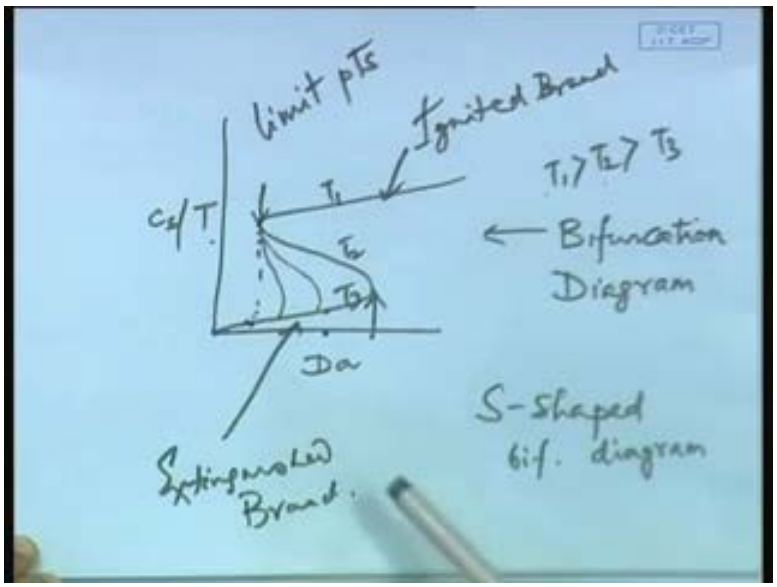


Now, again If you come back to this and here we had, we have the concentration and the temperature, so what were we talking of? We are talking of in a physical sense that

within the reactor, it is possible at a particular dam colon number for three concentrations to coexist, and that is the that is the origin of multiplicity, you understand what I am saying. So, in a CSTR for example, you can have the CSTR for any particular dam colon number can coexist in three different three different concentrations or temperature, so this is temperature then this particular dam colon number, you have temperature 1, 2 and 3, that is one possibility.

The second possibility is that the same CSTR, within the same CSTR these three temperatures coexist that is another possibility, so one possibility is that the entire CSTR exist in temperature T 1 or temperature T 2 or temperature T 3, with T 1 being greater then T 2, being greater then T 3, T 3 so the entire CSTR could be in temperature T 1 or T 2 or T 3. The second possibility is that within the same CSTR, you can have regions with temperature T 1, temperature T 2, and temperature T 3 and that is where these issues have in stability, the issues of multiplicity, the issues of reactor run away come in, because you have and then I explained you in the other class.

So, with that, so this was sort of a prelude and the reason we did that was because, substrate inhibition or is a is a sort of catalysis, it is an inhibition but, you can have a catalyze also say it is an auto inhibition or auto catalytic effect. So, and that was the basis of all these discussion and one of the things that, we tried to point out, while through this discussion is that even the simplest reaction which is, a going to b auto exothermic is an auto catalytic reaction, so this is not so uncommon, and then you will start to see that, what happens in a substrate inhibition, and I will show you in a minute.

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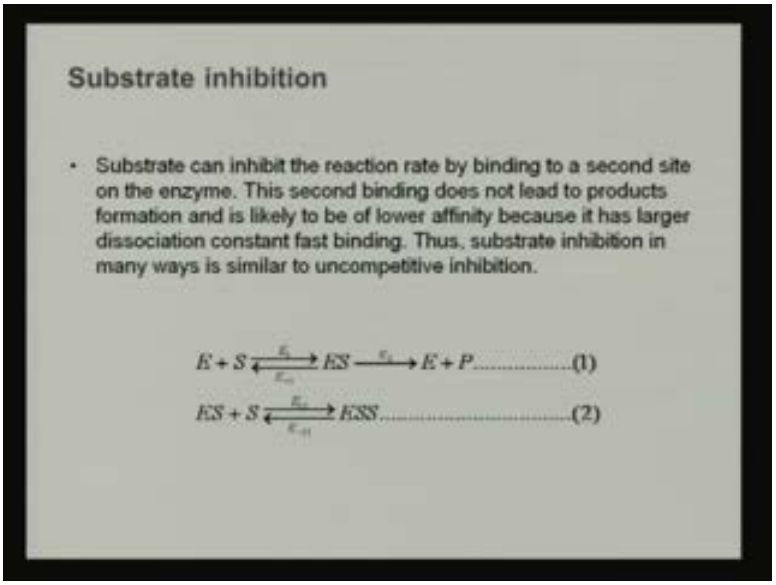


So, essential in a substrate inhibition, what happens is the substrate can inhibit the reaction by binding to a second site on the enzyme. So, you if you have the substrate S and then it can binds E S is being formed as a complex and then it binds to a second site of the, on the enzyme to form a complex what would be, so S is there its already binding to E S, and then the substrate is going to bind to a second site on the enzyme, so what would that be (No audio from 10:12 to 10:22), what would that be is very you know very similar, is as I said here it is written similar to uncompetitive inhibition, so its ESS.

So, the first reaction as you see here is same as what you had before that is E plus S giving E S right reversible and then E S giving product plus enzyme, back the enzyme, so that is there but, there some of the substrate can bind to a second site on the enzyme, so if the substrate is such that, it can bind to a second site on the enzyme then you have E S plus S giving a complex E S S.

So, what is what is the difference between these two bindings that is written here that, it second binding does not lead to product formation only the E S can lead to product formation a, and the second and the second point is typically has much lower affinity and it has a much larger dissociation constant there, is a much larger dissociation constant and lower affinity and that is obvious, because once you have once molecule is a substrate binding to the enzyme its very obvious that the second second molecule will have larger dissociation constant and lower affinity.

So, this is this is what happens, now it is hard for you I know, at this point to kind of connect what I am trying to say here, what with what I said before but, as we go on and start doing the derivations which we will do in the next few you know minute, you will start to see that what the connections are, so are you, sure are you good with this, so I will go on to the next slide.

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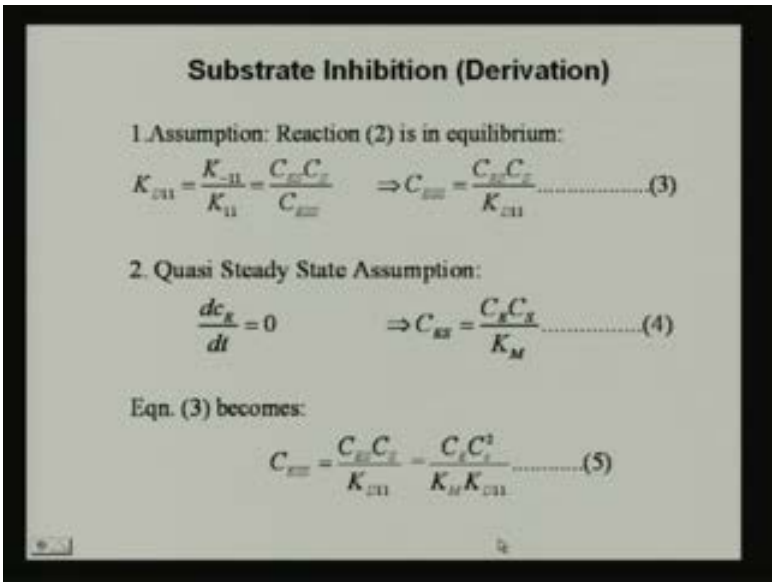


So, in the derivation what would be my you know yesterday I had discussed in great detail, so what would be my points to look for first is, what are the reactions that attain equilibrium right, so what are the reactions here that attains equilibrium?

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What what

E S plus (())

E S plus S equals not not both, so the second reaction only attains equilibrium, now next question was what?

Species (())

Species that attain quasi steady state, so that is the which one is that E S

E S

And the last point would be the constraint equation, so let us quickly write those down, so the first one is reaction two attains equilibrium and you could write the equilibrium relation (No audio from 12:43 to 13:20) then, so reaction two attains equilibrium, so you can have E S S as C E s C s times C s over K D. Second is the quasi steady state assumption for C E S, so this is this is typed over here it should be d c E S not d c E d c E S d T equals 0, which gives you C E S equals C E times C s over K M, so what you can do is you can now, substitute equation 4 in equation 3 and then you could write here C E S S as d c E C s squared over K M, do you see that, so this is where the non-linearity comes in right right clear.

Because, it C E S S equals this C E C this C s times C s over K D and C s you substitute over here, so you will get this C E E times C s square here, and that is where the first non-linearity comes in now, so this is what we got right, all of you go to the next slide.

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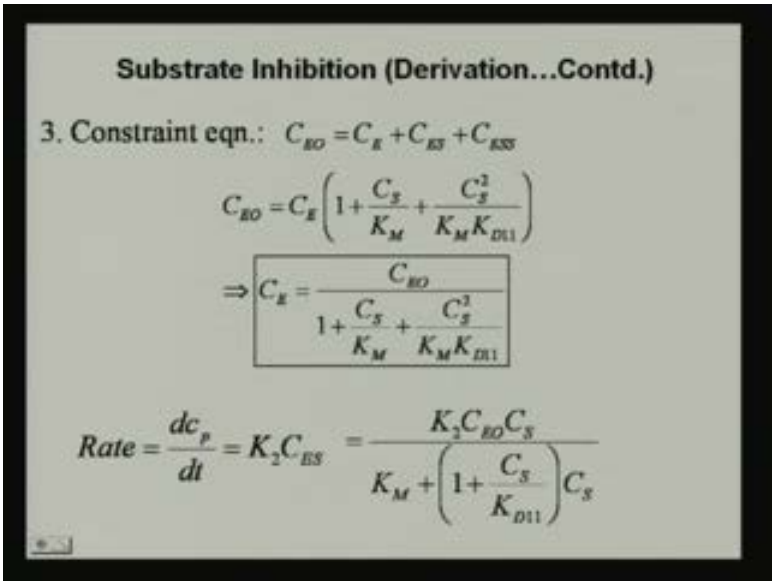


So, next point would be the constraint equation, so what would be my constraint (()) equals C E naught fine, so C E plus C E S plus C E S S equals C naught, so enzyme in the free form and the two complex forms equals C E naught. Now, we can substitute back what we had in the other two, and the other two, so equation, so C E naught equals, so once you substitute back you get C E naught not equals C E plus this term or in other words C E equals C naught over this term the denominator contains, first order and second order terms are yeah of C E S fine. So, what is am I rate now, what is my rate,

rate of production of P (No audio from 15:21 to 15:35), simplicate to times C E S right, so that you can substitute from here, because now you got C E terms of C E naught, 2 times C E S which is K 2 times C E naught C E S over this terms, so this is what you get. So, what is different between this rate that we got here, and everything that we did before?

Harsh, what would be the difference between this rate that we got here, and everything that we have done before.

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So, yeah that I have already said, so what

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Yeah but, in other word what that means is that it is not possible to expresses in the Michaelis–Menten part, so this is not Michaelis-Menten kinetics that is the most important thing, so substrate when you have substrate inhibition it is no longer Michaelis-Menten kinetics, that is what I want you to notice at this point. So, what do you know if I ask you to kind of intrude, what would you intrude to be how the rate go like in the Michaelis-Menten kinetics.

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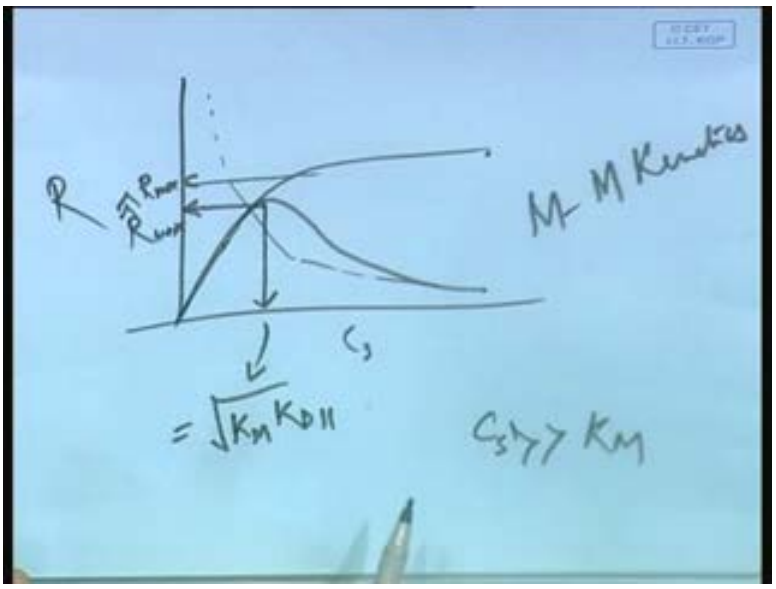

The rate goes like this, right, so it has an initial slope and then it reaches the, so this is the R verses C s and so this it reaches the R max right, so this is the Michaelis-Menten, what would you think how the, how this one would look?

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Like this

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Same then

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It will go down, like this, what do you think, say that here who wants to take the start

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Same

Shrikant, what do you think

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You have the rate expression in front of you on the screen here, and all you need to do is, so it is a function of C s, so all you need to do is tell me how this goes as a function of C s.

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Yes I think what you said is right, that is how it looks like, so what is this peak going to be what is the rate, so this is the maximum rate now, so that is the difference earlier the saturation limit (()) limit that you reach over here was the maximum rate, but now use the maximum rate over here, so this is, it is called as R max hat or something. So, R max double hat, so this maximum rate is different, now the earlier maximum rate was easy to obtain, how did you obtain the earlier maximum rate you in the limit of C s much much greater then K M right; so you just put C s much much greater then K M in the equation and you got it.

Now, how do you obtain, because this is the quadratic form, so what you have to do is you has to, you have to take a derivative of that and equate it to 0 to get the maximum rate, and what you will figure out is that the maximum rate occurrence at C s value which is equals here, equals square root of, so when you do the derivative, take the derivative equate to 0 do the rest of the algebra, we will skip, we are skipping that here a little bit of algebra is there.

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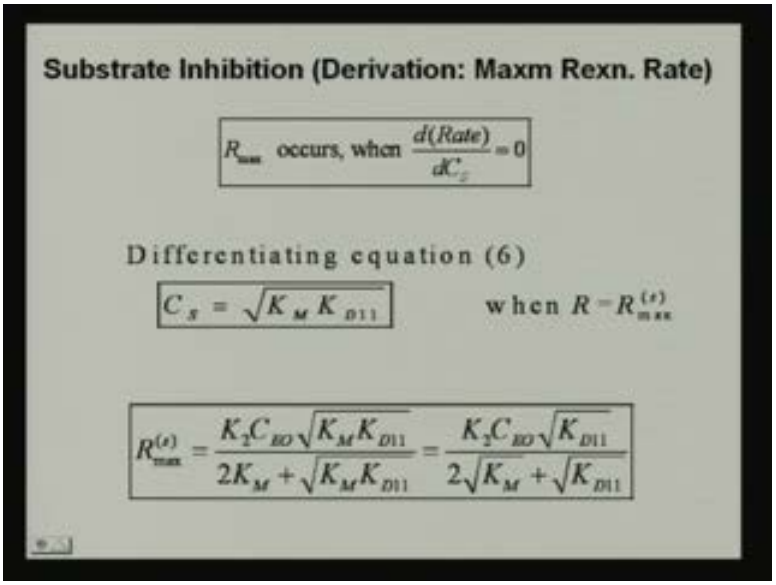


When you do all that algebra you will figure out that your C s equals square root of K M D I I acts C s equals square root K M D I I K D I I you have the maximum rate, that is R equals R max. And the maximum rate itself when you put that value in value C s in to your R R you get the maximum rate at this number, which is dependent on the all the rate constants and C naught (No audio from 20:32 to 20:48) right, so we will move on.

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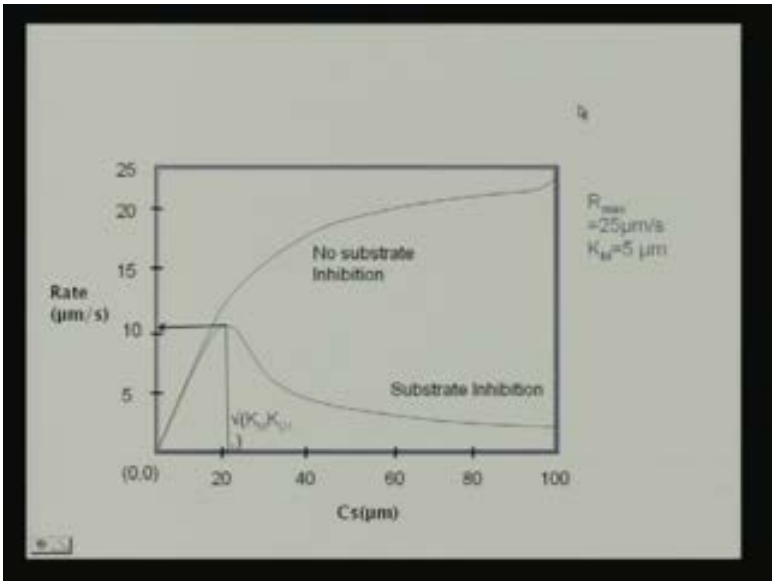


So, what I will show you next is, the plot, so this is the plot of substrate inhibition without substrate inhibition on top and with substrate inhibition below, so as he correctly mentioned that without this Michaelis-Menten kinetics, without substrate inhibition it goes and actually it should saturate. And with substrate inhibition it goes up, up to a certain point it is an it is a same, why is it the same can somebody comment on that, why are these two slopes more or less the same.

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Then what happens

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Right when C s is low then the effect of inhibition is a substrate inhibition is not there, because in the limit of small C s the substrate inhibition you have, the formula for substrate inhibition that that you had, lets go back to it here, we will go back to the old formula without inhibition; so with and without inhibition makes no difference as small value of C s, because C s square is very small, so yeah these two lines merge and then they reach a peak which is given at the value of square root of K M K D 1 1, and then it goes down again and goes to goes to, what how does it goes, go to? See here, you could see here you know for example, if C s is very large then it will go to 0 right.

Because, what was happening was see mathematically what was happening before was that, the numerator and denominator were both first order, earlier were both first order in C s right as a result it cannot go to the (()) large value of C s it will go to saturation limit whereas, here the numerator is first order in C s and the denominator is second order in C s where C s goes to a large number it goes to one over C s and therefore, goes to 0 fine.

So, this is about substrate inhibition, so we are more or less done with inhibition, so the kind different kinds of inhibition we studied competitive non competitive, uncompetitive and substrate, so well what we will do today was its a rap on the enzyme kinetics as we are studying in this class.

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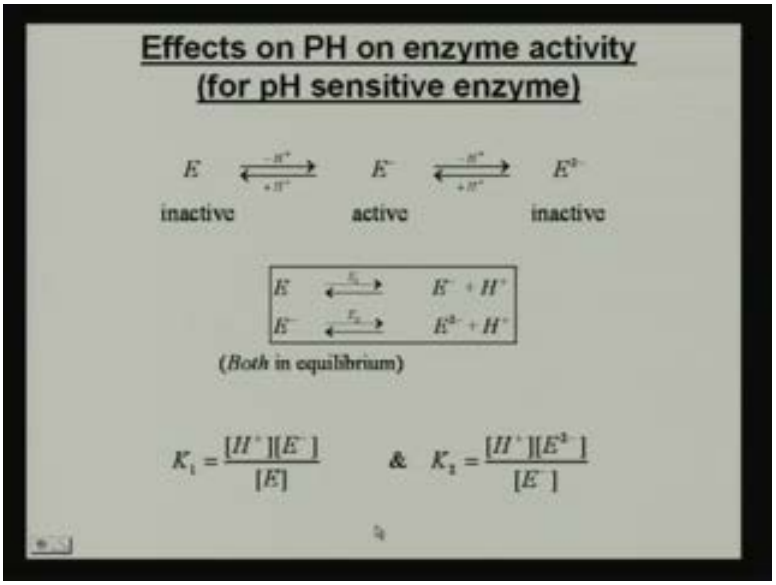


And the final thing two or three things that we are going look at are first is the effect on p H on enzyme activity, so it turns out that the specific kinds of enzymes work within a certain p H range, so you know you know this so what would what is the P H range of the human body?

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7.2 to 7.4 that that is how it varies, and depending extracellular and intercellular and so on, but over all range is 7.2 to 7.4, so as you will understand that many of the enzymes are most of the in enzymes that physiologically enzyme that word can between a human

system would have to be active within that range right, if its activate 5 or 6 or 8 does not make any sense.

Because, the range the window that we have is that and enzymes you know one of the ways that that the specificity of the enzymes work is the p H, so when its sensitive to p H or sensitive to a certain range of the P H enzyme is specific, so if you take the enzyme outside that range it would stop working, so one of the things that you know if you are doing experiments in the lab is to control the p H using buffers and so on, and keep it at a certain within a certain range where that go where the enzyme is active.

So, what happens when enzyme is not active or you go beyond the P H range where it suppose to be active, so I will show you this, so this is what happens, so this you know what happens is, because of the effect of p H there is a proton transfer, so because of the effect of p H there is a there is hydrogen ion transfer and the enzyme is typically a huge reactive in this form where, which is the E minus form and then if there is a there is a hydrogen ion transfer to it and then it can become the E form which is without the nonionic form or it can go to the double (()) both of which are inactive.

So, it is active within this range, so essentially that is how p H effects, so excess of hydrogen ion it will go from the E minus to E form depletion of hydrogen ion it will go from the E minus to E 2 minus form, and so it will be it will be active only in that range in the in the E minus form, so that is how it is it is sort of.

So, when I am trying to figure out, so at any point in you know at a certain p H for example, can you expect the entire enzyme that you put in there to be active no right, its only a fraction of it that is even its at certain certain p H, so even if you want, if the enzymes range is 7 7 to 7.4 and you are the p H that you are maintaining in your solution is 7.2, but still 100 percent of the enzyme is not going to be in the active form, a part of it are going to be in E form and part of it are going to be in E 2 minus form.

So, how would we figure that out, how would we figure out, how much of our enzyme is actually in this in the active form, so what we do is we assume that these two reactions, this is the first reaction which is E going to minus plus H plus, so H E minus is here active enzyme, so a proton is added to it and it goes to the E form and K one is your equilibrium constant, for this.

The second one is, E minus again is in the active form and a proton is taken out and it goes to the E 2 minus form and K 2 is the equilibrium constant for this right clear fine, so all we got to do is now write the equilibrium relations for these, because we need to figure out that what is the fraction and which these this is distributed the enzyme is distributed between one active and two inactive forms.

So, we so we write the equilibrium relations for these two reactions right, what should we do next what is the intuition tell us?

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What is that?

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What what does the intuition tell us, what should we do next

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Take ratios of two but, what would you do, you would you cannot eliminate, so what do you want to find out, so let us first answer that question what do I want, what should be my goal, what do I want to find out in this?

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E minus amount of active enzyme out of the total amount of enzyme that is there what is the fraction of the active enzyme, so if you would if you would divide one by the other or multiply one by the other what happens nothing happens you know, if you divide one by the other the hydrogen ion but, you have the you have all three left, if multiply one by the other you got rid of E minus, the one that you want to figure out, so that is not the only, that is not the solution essentially, so what what other option is there and you have to take a clue from what we did before the answer is there.

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Right, so what is the constraint equation?

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Right, very good

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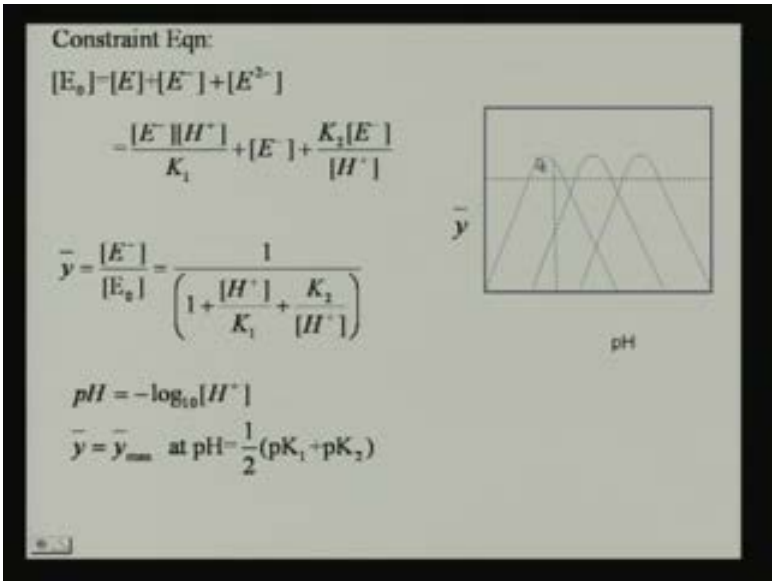


So, that is what you have to do, so you can take ratios and all that but, the most important thing at this point is to be able to write the constraint equation, which is that the total amount of enzyme that is present is distributed in three forms the E that is E minus that is a active form, and the two inactive form that is E 2 minus and E right, so that that is what you need to do.

So, once you do that then what you can do is like you did before, is try and express everything in the form of E minus, now use equilibrium relation, so express E as a function of E minus, express E 2 minus as a function of E minus right, so you can take E minus out out of the parenthesis and you will have, so E naught equals E minus times this sole number 1 plus H, H plus over K 1 plus K 2 over H plus fine. So, this is your this is your fraction of the total enzyme, that is active good, so that H plus is something that, I do not want to use in this form I want to use it in the form of p H right.

So, what I do is simply use a definition of p H as minus log H plus log to the base at 10 10 H plus and therefore, I can just rewrite this expression that I have over here y y bar in terms of p H right, should not be a problem, so there will be a negative and a positive sign, and now before I go should explain what the, what I wrote at the bottom, let me show you the plot.

So, this is how how the plot of y bar versus p H look like, why this plot again yon know, so what is there what you see there there is a nonlinearity over here right in H plus, if you take H plus for example, in the numerator what happens in the denominator you get H plus plus H plus square plus constant and in the numerator you get H plus.

So, again there is a nonlinearity out here and as a result of which when you take the fraction for example, if I take at a certain fraction I can look at it two ways, so if you look at the line over here the top line, the horizontal line at a certain fraction I obtain see see easy to measure I can obtain what is the amount of amount of active active (()) enzyme that I have in the system, I can measure that and at that fraction I am trying to figure out from the fraction what is the p H, but the problem is am unable to the reason is this is a repetitive curve, this is a non-linear curve, which is multiple solutions again.

And then at the particular y bar I can have several values of p H, so this could be one value, this could be another value, this could be 3rd value, 4th value, 5th value, 6th value and so on right, again if you look at the other way at a particular, so this is a vertical line now, at a particular value of p H, if I want to figure out what my fraction of enzyme active enzyme is, I have, I can have two solutions right, I can have one solution also but, within a very very you know specific places I can have one solution, like a place like this, where the two intersect, but otherwise typically here I have two solutions, here how many solutions I got, three solutions, if I draw the line over here, so 1, 2, 3 again here, 1, 2, 3.

So, most of the K places you see I will have three solutions in some small regions windows I can have two solutions, in some very very small windows I can have one solution, but as the end of the day this is again a question of multiplicity, so when I, if I give you particular value of the fraction of enzyme and ask you to find what the P H is then there are n number of solutions. If I give you p H and tell you what the fraction of enzyme is typically there will be most of the cases there will be three solutions, so this is that that these are the reasons you know I talked to about multiplicity and and bifurcation, because unless you understand that theory would be hard for you to appreciate what is going on here and this would like look like (()) solution.

Now, what is important is that, you have to draw your window over here right, so your window if it is a that is in in the physiological system for example, if your window is 7.2

to 7.4 then you draw your window over here and try to figure out what your fractions are but, again in that case you will get multiple solutions, you can have multiple values of the fraction. So, what it means that even at a certain p H you can multiple values of the active enzyme can coexist even at a particular p H, so this is these are some realities that we have to live with you know there is no way to walk around it, so this is this is a concept of the of effect of p H.

So, you more or less understand how it how it effects you know in a general trend, this is a general trend is that it increases with a certain as you keep increasing the P H then it attains the maximum and then it decreases but, that as I said is a very general trend, the reason is that would be a very narrow way of looking at it.

Because, you see this multiple solution that are there and as a result of which you cannot makes of generic comments you understand what I am trying to say, so you if you take one of these mountains and then say that as p H increases these y the active enzyme increases and then reaches the peak and decreases, that is a very narrow way of looking at it, because you these have other overlapping mountains, overlapping other mountains overlapping with them, so you cannot make generic comments, so you have to understand this from a from a broader point of you.

So, that is about it with the effect of p H well yeah, so at the maximum, so this value that is that is attained the y max the maximum value that is there and that is what you would like right for your system if you want your system to be not inhibited, if you want most of your enzyme to be in active form, so the maximum value is something that you would like to attain and that maximum value is attained at a p H of p K 1 plus p K 2.

So, if you are doing experiments and you want to outside the body, so inside the body of course, you do not have much control over p H, but if you are doing experiments and you want to maintain the p H then you try and maintain it around around a value which is half the p K 1 plus p K 2, so which began and K 1 and K 2 being the equilibrium constant for the two reactions, which are not fine right, shall we move on.

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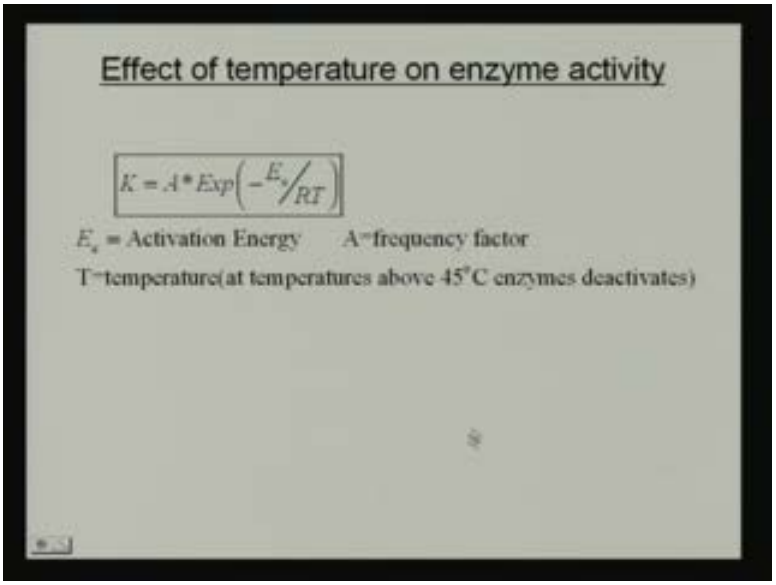


Next is the effect of temperature and that is a very easy effect actually, so the on the enzyme kinetics, so what happens is in the effect of temperature is at the rate constants that you have for enzymes, it is you know they just follow (()) kinetics, so K would be the (()) factor times exponentially minus E O R T, so this is a very straightforward thing. And but, what is important to notice over here that, these enzymes are typically active over a certain temperature range just as a human physiological body p H we discussed and the and the range for that is between 7 7.2 to 7.4, so the human body also is a temperature range, so what is it normal temperature.

(())

How much

(())

37 degree centigrade, which is what the body tries to maintain, so even if you are in very cold you know if you outside the temperature minus 10, if you are in a very cold country your body will still try to maintain 37, that is one the miracles of the body, so it has all these cooling effects and and heating effects, internal cooling effects, internal heating effects and then you know, so people the body will try to maintain the temperature, but it cannot always you know in this winter itself probably around 500 people died in India just from cold.

So, the body tries to maintain that temperature as long as it can and when it cannot obviously it gives up and the other end you know you hear during some more people dying from sunstroke, so that is another extremity, so the body can maintain so 37 is the optimum temperature it can maintain, it can take temperature up to 48, 50 where up to say 48 or so 50 may be, where what happens is that, the body tries to maintain, so the internal temperature is always maintained around 37, and if it is not maintained around 37 then what you have, you got fever right, that is why.

But, if you look at say even when somebody has a fever of 102 or 103 and and you take the deviation from 98 or 97.5 or something you see the deviation is only couple of degree, so 37 and you can go up to 39 or 38.5 and then couple of degrees yes so the temperature range within which the body work is very small. Now, enzymes obviously would correspond to the temperature the physiological enzymes, enzyme that work in a human body would try to correspond to the temperature range, is called and if the temperature range is higher than that, it still takes it to some extent, but above 45 even this some of you, if you are doing experiments with enzymes you will figure that is above 45 degree centigrade, the enzyme start to deactivate, so this when you talking of effective temperature they are valid within a temperature fine.

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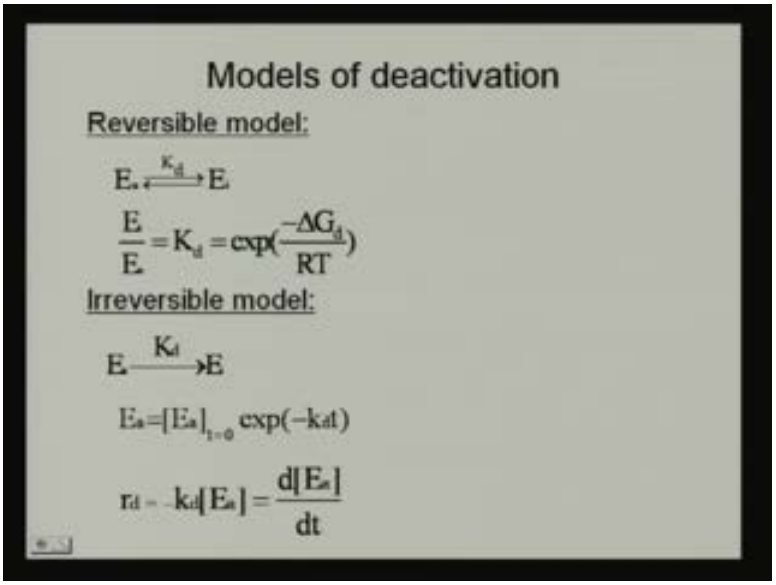


Now, now next thing we look at is a effect of deactivation, so at temperature is higher than 45 we said that it deactivate, no what is what is the mechanism of

deactivation, so what you know if I ask you question, what do you think would be would be the mechanism of deactivation or could be the mechanism of deactivation (No audio from 38.02 to 38.11). So, there be enzyme that is in deactivated form and there is an enzyme that is in activated form right, so by the way this is different do not confuse it with an inactive form, inactive form is form that is right now not active but, can become active at any point of time, so it is not deactivated, there is a difference between deactivated form and the activate.

So, there are two models typically people think of it in two different when ways, one is known as the reversible deactivation and the other one is the irreversible deactivation, so in reversible deactivation we can go to the deactivated mode and from that you can get back to the activated mode, where is in the irreversible deactivation, you assume that you go to the deactivated mode irreversibly and you cannot get back to the activated mode.

So, let us have a quick look at these two models, so first one is the reversible model, so and second one is the irreversible model, so the reversible model is something like this, it is a very straightforward thing, that is activated enzyme goes from the activated form to the inactive form, deactivated form. And so here, E I is you know subscript E I is used because, it can get back to the activated form, that is a reversible one, and so the ratio is given by the equilibrium constant, which is related to the gives free energy change in, the system from the for the transfer between these two forms.

So, the second one is lot more straightforward it is irreversible form, so straight away it goes from E a to E I (No audio from 39:52 to 40:02), and then you know the kinetics of it is straightforward, so d E a dt equals minus K d E a and you can integrate that to get this, it is very straightforward (No audio from 40:19 to 40:43), that sort of brings us to the close of this chapter which but, we will continue with enzymes with little difference.

And what what, so let me summarize for you quickly what we did, so essentially we started started with structures of enzyme and so on, and sorry so yeah forget this, I think what we did with we started with structures of enzyme and so and then talked about the Michaelis-Menten kinetics, and so talked about Michaelis-Menten kinetics and then you know talked about the quasi-steady state assumption figuring out how the Michaels constant are evaluated graphically here.

The slope and final asymptote, so the and look at the two asymptotic limits of small C s and large C s that is one thing we did and then we looked at different ways of evaluation using different plots, then we tried to figure out whether the quasi-steady state assumption is valid, and we established some conditions under which its valid.

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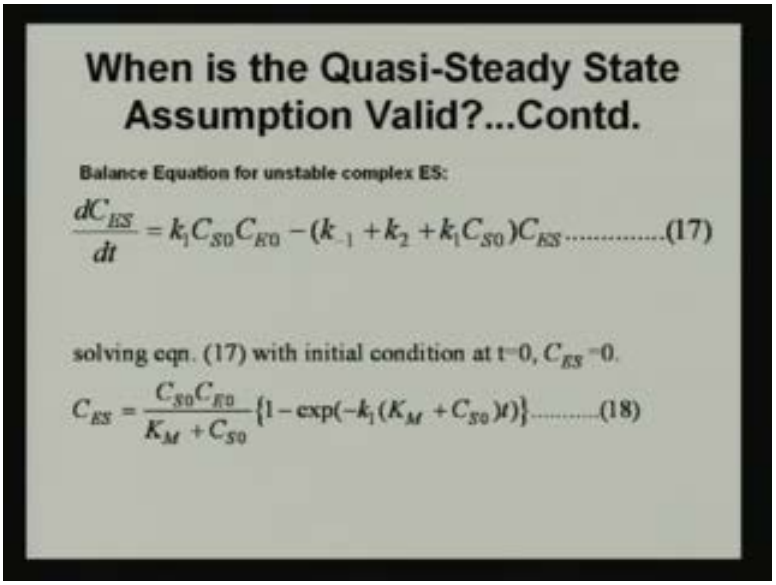


So, just to summarize you know, because some of you may not have been there in some of the classes, so we figure out that this is may neglect the quasi-steady state assumption in which case we have to solve this, dynamic equation and then we compared the rate constants of two process to figure out if that is work or not.

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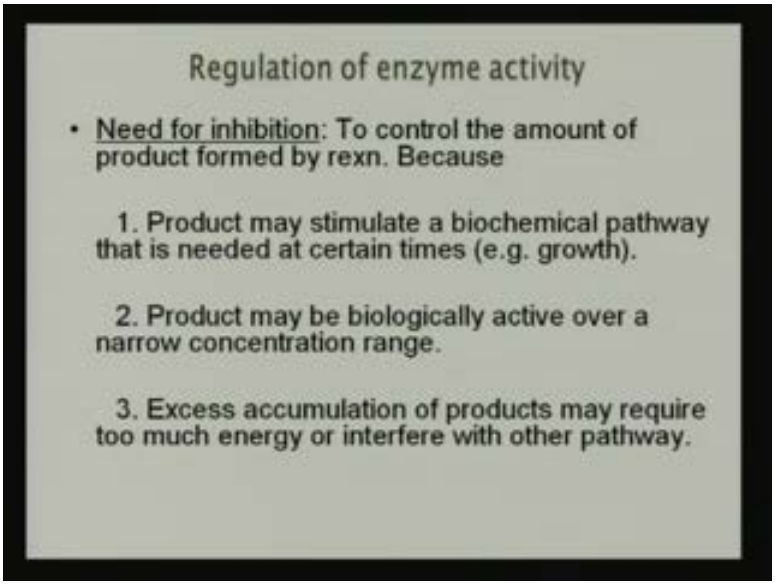


Next thing we did was, we tried to understand the regulation of enzyme activity using inhibition and we talked about why we do inhibition and we talked about several times and now in to the (()) and then we looked in to the different kinds of inhibition that you have competitive, noncompetitive, uncompetitive and substrate inhibition. So, what it looks like, so these are some of the examples, we did and what does it look like, is that for competitive, so in competitive inhibition if I am to summarize what happens is that the inhibitor and the substrate the compete for the attention of the enzyme, so both of them try and bind to the same active site in.

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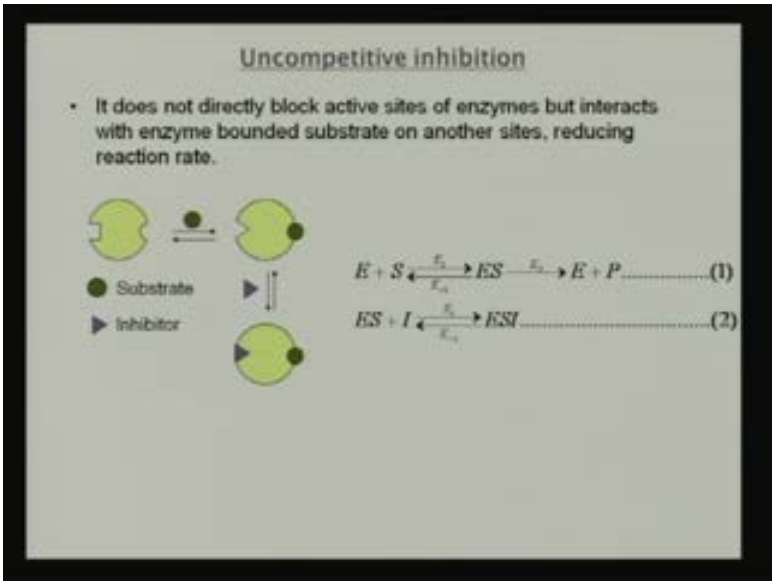

Next one we did was uncompetitive inhibition, in uncompetitive inhibition, the enzyme and the substrate do not compete for the same active site, so the enzyme the inhibitor and the substrate do not compete for the same active site and the inhibitor essentially binds to the non non-active site you know another site whereas, in noncompetitive inhibition. This is uncompetitive in noncompetitive inhibition both these things occur, so the inhibitor binds to the active site as well as binds to the non-active site, and I said this is a most potent form of the inhibition, because this is what happens.

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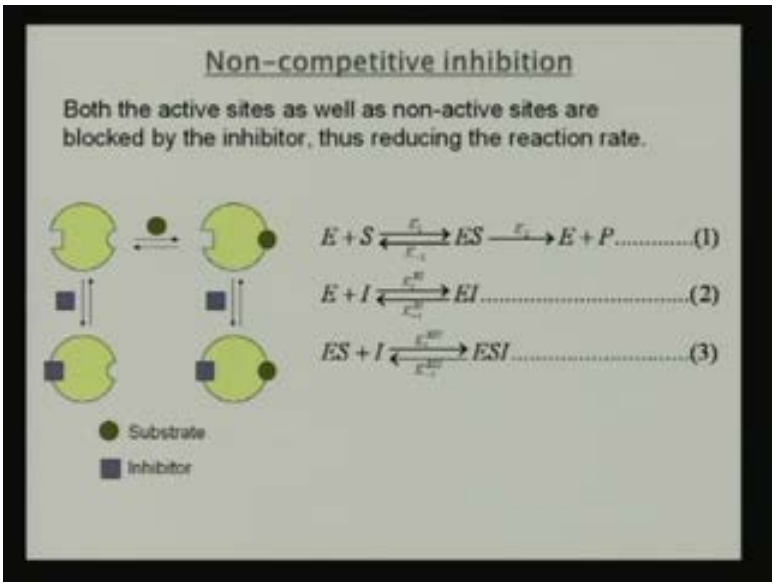


Now, the advantage with this kind of inhibition noncompetitive inhibition is you can do a couple of things; you can vary both the rate, both the slop and the R max and the K M in the slope and the asymptotes.

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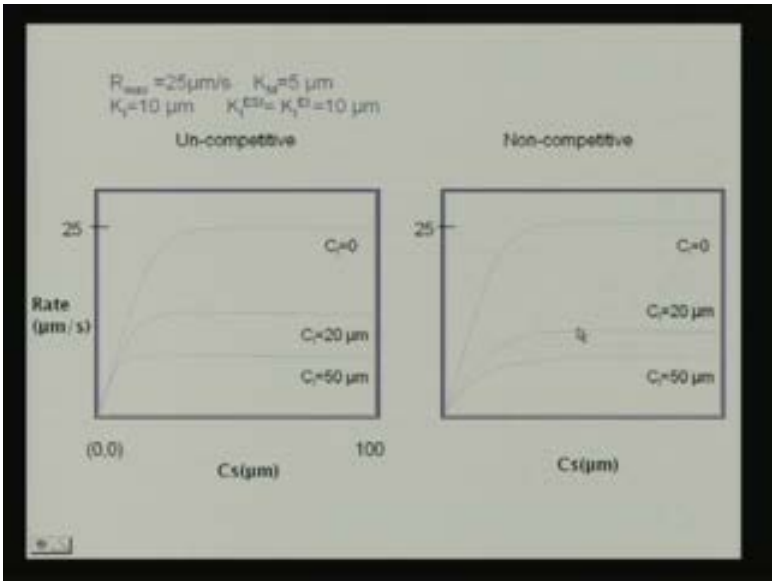


So, what happens in uncompetitive inhibition, if you look at this graph what happens an uncompetitive inhibition is that the initial slope is the same, because the ratios cancel out if you, so remember what we did in last the class in, noncompetitive both are different.

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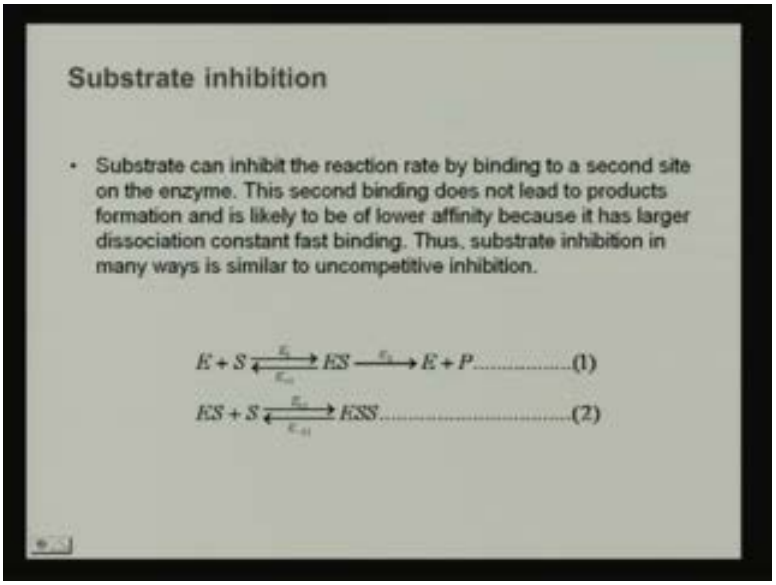


Then today, what we did was substrate inhibition and what we figured out substrate inhibition is positively different from other kinds of inhibition, it is an auto inhibition, that is the substrate itself inhibit the system, and is qualitatively different, because the

earlier kinds of inhibition we looked at retain the Michaelis-Menten form, that is you had a certain slope, a linear slope and then you go over and reaches certain asymptotes.

In substrate inhibition it does not retain the Michaelis-Menten form it initial rate slope is still the same, but it goes and reaches the peak then it comes down, so I will show you that picture, here and the peak is attained at a certain R max which you can calculate, and then we quickly looked at the effects of the p H, effects of temperature, effects of deactivation. So, this is, this part that we end here, but if you have a quick question, one of you quick question on this, I am ready to answer is there is any then.

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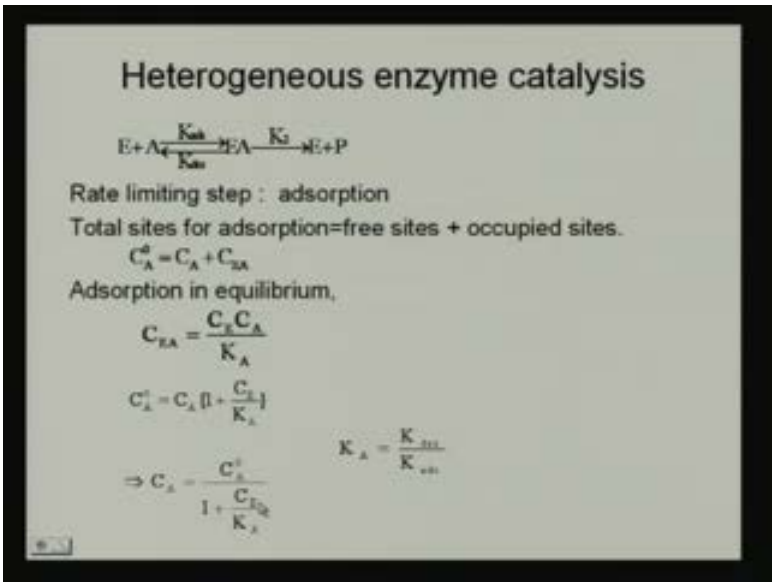


If there is none, then we move on, we will move on to the next chapter, and so what you know one of the things I want to impress upon you after doing all of these is the fact that this enzyme catalysis and enzyme kinetics that we looked at, are in which which medium what what is the, which medium the enzyme is in and which medium the substrate is there and so on.

What phase, what is a phase

(())

And

(())

No, the calculations that we did till now

Both in same phase

Both are in same phase right, yeah so both are in liquid phase not (()), specifically to view (()) the same phase, specifically both are in liquid phase. So, is the Michaelis-Menten kinetics not valid if what he is saying that, if one is in solid phase and one is in liquid phase it is valid yeah, it is definitely valid if one is in solid phase and one is in liquid phase. But, there are some other effects that, that come in, so if both are in solids liquid phase for example, then we take in to account the reaction fine, if one is in solid, one is in liquid phase, the reaction kinetics still remain the same and what we studied is still good right.

What in addition to what we studied, what you need to consider say for example, the enzyme is working on a on a solid spherical substrate, the food you eaten for example, is trying to digest or you know something some glucose is trying breakdown or some something some sugar is trying to breakdown for example, and sugar you can say solid phase. And so what what would be different, now you do not get confused I already said that all the reaction that mechanism that we studied are all good still, but in addition to that what phenomena kicks in.

Yes Aruash, you done this is in the lab, so you know if you have sugar and you trying to you know break it down in cellules and the sugar is in solid phase, so if sugar is in liquid phase, so the two possibilities right you can take the sugar dissolve it in water get it in to liquid phase completely and then you add your enzyme to the liquid phase, you have, you can perform your experiments and you get results similar to what we showed now. If you have the sugar in solid phase and you add the enzyme liquid phase enzyme for example, on the solid phase.

What, how what would be different now, phenomena wise what would be different?

Enzyme (())

Yeah then, that is right yeah enzyme was (())

(())

And what else

It is (())

Diffusion in to the substrate, if the substrate is in solid solid, so two things both what you said and what you said, so if it is you know if adsorption is a is a possibility if it is a heterogeneous, so when we talk also this what happens is now the title of the name next next slide, that I have over here and that is a beginning of the heterogeneous next chapter, but it is like a interlude between this chapter and next chapter, and how we connect.

So, what we studied till now was homogeneous catalysis homogeneous catalysis means that both the catalyst and the reactant are in the same phase right are in the same phase, and typically in to the fluid phase, so that is homogeneous catalysis, heterogeneous catalysis is, if anyone of these two is in different phase from the other, then you will immediately become heterogeneous catalysis, does not have to be that both are in the solid phase. If any one of the reactant or the catalyst are in different phase from the other then it becomes heterogeneous catalysis, so when it becomes heterogeneous catalysis, then there are other things that kick in, two things that kick in and both what he said one is an absorption effect and what he said one is the diffusion effect right.

So, first if, first you looking at the enzyme on the surface, so if you looking at the enzyme on the surface then enzyme is coming and to the surface of the substrate the enzyme, let us assume that the enzyme is in liquid phase and substrate in the solid phase could be the other way around also, but let us assume one thing, so the enzyme comes in and sits on the surface of the substrate. And is there absorb in the surface of the substrate starts to react, then post absorption, so but this is only the surface and the substrate is they say large round body, so it has to diffuse in to the core of the substrate, so post of post that if there is a diffusion right; and then what about the product, these are the things you should should say tell me, I should not be telling you, because these are the things you studied in the heterogeneous catalysis, you studied in reaction.

So, what is the step, tell me, what are the steps? One by one

(())

No, first absorption

(())

First absorption a

(( ))

No first absorption, then diffusion in to the pores then reaction third, then fourth diffusion back

(())

No, diffusion back to the surface out and then desorption, so these are the five steps. So, adsorption first, second diffusion in to through the pores, third reaction diffusion out of the pores, fourth and fifth is desorption, this is the mechanism in which its it goes on. So, here what I am you know this is a simplified form of this and I will go in to much more detail, but today we cannot finish, but starting next class, but this is a simplified form whatever I done over here, I discounted the two diffusions, the diffusion in to the pores and the diffusion out of the pores.

So, I am just assuming it is not even a porous solid, this is a solid just a simple case where there is no not a porous solid just a solid on the surface and the reaction is occurring, but just a basic of heterogeneous catalysis, we will go in to diffusion in the next few lectures make no mistake, but this is a very simplified case where there is no pore diffusion nothing like that is happening.

So, this is a solid is here and the reactant is coming and and and the enzyme is coming and reacting with the absorbent and forming E A, so this is sorry its absorbing, this is the absorption E A and then E A leads to the product formation this is this is the reaction, so a E I used, because you know just to signify that is in in in a different phase rather than, so and the absorption is assume to be in equilibrium, absorption is here its reversible, is a reversible form and then the product formation.

Now, typically what happens is the in most cases it is a absorption that is a slowest step, not the reaction, because if you look the rates of absorption and you look at the rate constant for reaction typically in most cases it is a absorption, that is it slowest step, so

the rate limiting step in most cases is adsorption; now there is a constraint equation that you can write here, as well like before and this is as I said this is a sort of interlude between the next chapter and last chapter and we have not started the next chapter. But, just for a sense I am trying to connect what we did before and what we can do later is that you know, you can come up with some sort of a constraint equation here, as well and what would that be, one of the things you can tell me very easily that, the total amount of enzyme in the free form and the (()) form is a constant.

But

(())

Rate of

(())

Rate of product formation not rate of reaction, rate of product, if that is that is a good point yeah, so if that is a rate limiting step then the rate of product formation is the rate of adsorption, now as I said before that the constraint equation could be that, the constraint equation could be that my total amount of enzyme in the combine form and this free form is a constant, but if your adsorption is a rate limiting step is would would that be the kind of constraint equation you would want, no.

Then what would be your constraint equation? The constraint equation would, now been adsorption, because that is a rate limiting step see, what is happening is that the number of active site that it can provide is that is the rate limiting step right. So, what you want to do is something like this you know, so what I have on the screen now, so the total sites for adsorption is equals the free sites plus occupied sites, and the total site for adsorption for us particular system is known. And you know so you can have the C A naught equals C A plus C E A and adsorption if that is in equilibrium, then it is assumed to be in equilibrium, then you can have get a relationship between the C A and the C E A fine.

And quickly I have just a minute, so I will quickly go through this rest, so because this is easy, so once you once you have understood this is the rate limiting step, and then this constraint is going to be an adsorption, so total number of sites equals, free sites plus occupied sites and then you can write the constraint equation that C A naught equals C A

C A plus C E C E A and you could write the equilibrium relation, so then you can go back like we did last time, go back and substitute the equilibrium relation over here and get a relationship between C A naught and C A and C E A right.

And K A here is the adsorption equilibrium constant this is the desorption rate constant over the adsorption rate constant. And the final thing you will get is C A now, that is you will get in terms of free site; you will get in terms of the total sites and the amount of enzyme. So, I think we will stop here today, and little bit is left, but I do not want to hurry you through the thing, so we will continue in the next class on the on this, and then move on to the next chapter, thank you.