Advanced Chemical Reaction Engineering
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Lecture - 36
Reaction Engineering Examples in Biochemical and Environmental Engineering

We talk about Biochemical and Environmental Reaction Engineering.

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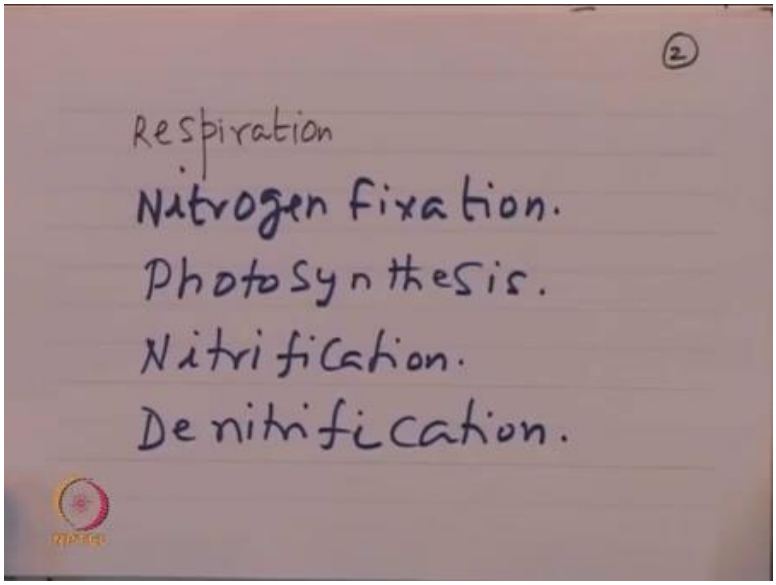

And it is quickly look at what is the look at respiration, nitrogen fixation, a photosynthesis, so nitrification, de nitrification. Now, all these reactions are well known to us. And there are the regulated by micro organisms and enzyme are most important part of the worth of the micro organisms do. So, what we will do know is quickly look at, what do enzymes do and how the enzyme kinetics looks like.

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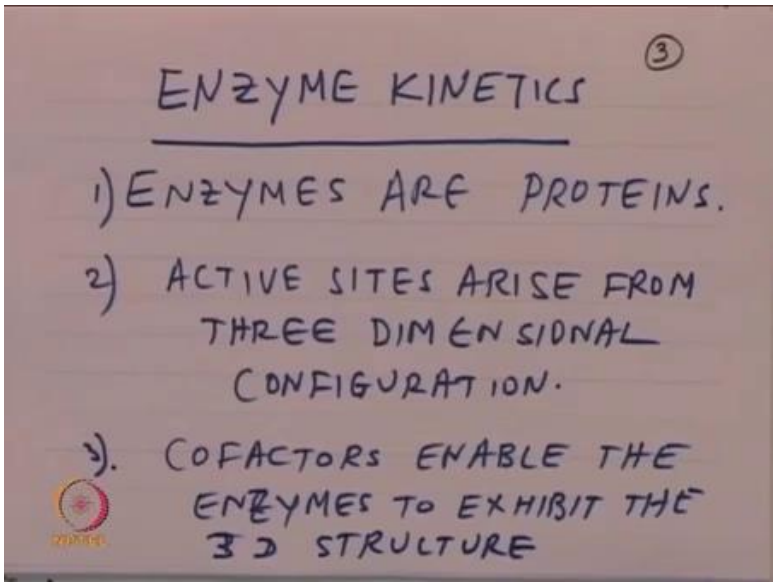

So, first thing we want to do is enzyme kinetics. Now, it is all known to us that in enzymes of proteins. So, enzymes of proteins, they are proteins that is 1. 2, there active sites the arise from the way the fold in space, phase active sites arise from three dimensional configuration. Third thing is, this configuration, that these enzymes are able to achieve is when you add certain cofactors. Cofactors enable the enzymes to exhibit the 3D structure. These are the three important features that enzymes are able to do.

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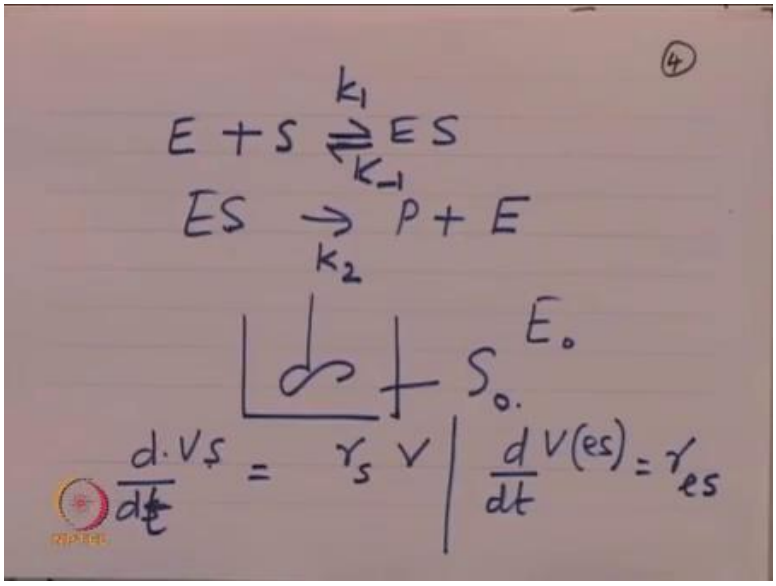

So, what is done in the enzyme literature, that if we have an enzyme, which has it is

active form, it forms here complex. And this complex the reacts to form enzyme products plus the enzyme. Now, enzymes are products of biological activity. But, it is now known that enzymes can be extracted purified and used outside the living systems. That, we are able to do in many cases.

Now, if I call this as k 1 call this k minus 1, call this is k 2 it can write, suppose we perform this reaction in an equipment, which has the substrate S naught, it has an enzyme E naught. Then, you can write you are material balance for the substrate as r times s times v. This is something that we all have written. Similarly, you can write a material balance for the enzymes substrate complex, this material balance for the enzymes substrate, which is the material balance for the enzymes substrate complex.

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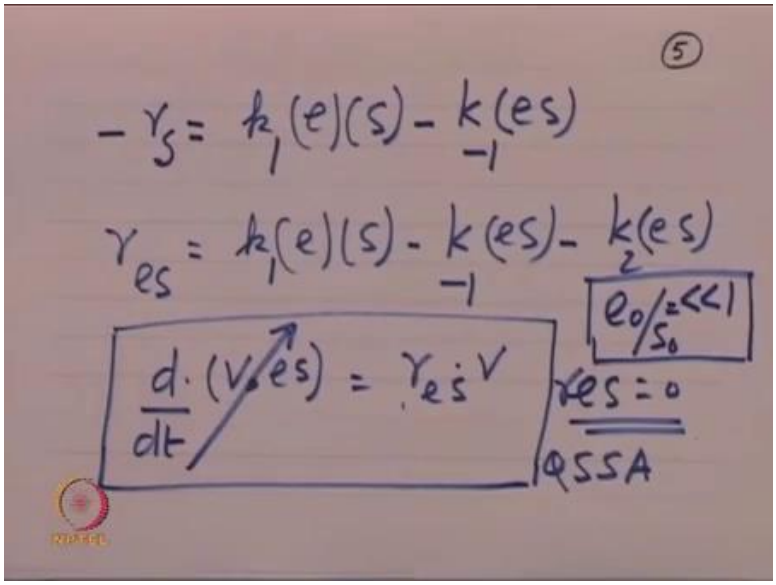

So, let me write all these functions properly r s. Please we note r s, ((Refer Time: 04:57)) r s rate of formation rate at which get consumed. And then, r s is I am write minus apart k 1 e times s minus k minus of 1 upon e s, ((Refer Time: 05:15)) r s is k 1 times e times s minus 1. Now, r e s that means, rate at which ((Refer Time: 05:25)) the enzymes substrate complex is get in formed, I will right here. So, it is k 1 e times s minus of k minus of 1 e s minus of k 2 times e s is notice here.

Rate of formation of e s complex is k 1 times e times s minus s minus s. Now, what is frequently done in the enzyme literature is that ((Refer Time: 06:01)) have try to V here and forgot write it V here. Now, this rate of formation of the e s complex is assume to be

this rate; that means, e s complex assumes stationary. On other words, e s remains constant, this does not change r e s equal to 0 is the assumption, which is quad s quasis the steady state approximation.

On other words, what we are saying is that, as the enzyme reaction proceeds. The complex does not change in concentration, remains reasonably constant. So, that r e s can be assumed V 0. It is an assumption that the enzyme literature may it is write through for a very, very long time. And in biological process it seems to be a good assumption. But, in commercial synthetic process it applies under certain conditions. The condition is the condition and which it applies is e naught by s naught should be small.

In this assumption is could, then this approximation is considered to be very good. On other words, we want uses approximation, we have to see that this ratio is quiet low.

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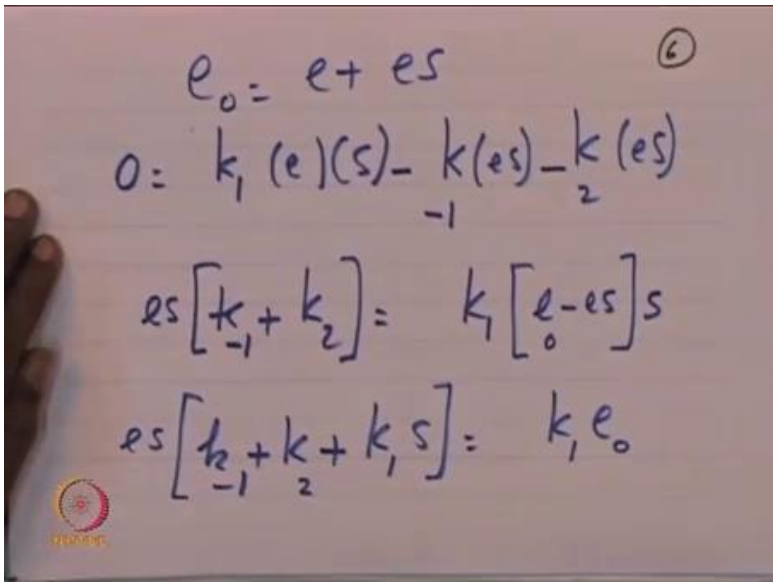

Now, setting this is 0. That means, 0 equal to k 1 e times s minus k minus of 1 e s minus k 2 e s equal to 0, gives you e s k minus 1 plus k 2 equal to k 1. What is e, e is simply e plus e s. Then, I will write this as e naught minus of e s into s. So, I will do a further simplification k minus of 1 plus k 2 plus k 1 s equal to k 1 e naught. I have just taken this on to the other site, e naught is the good question, good question e naught.

See, this start ((Refer Time: 08:30)) this enzyme reaction, which certain amount of initial enzyme in the process. So, it is the total amount of enzyme that we have added to the

process for initiating this enzyme reaction.

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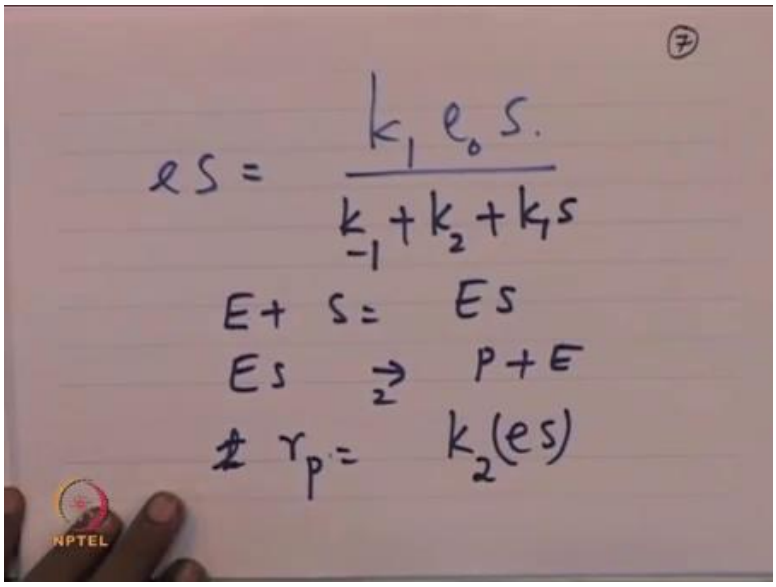

So, you get e s let us go forward e s equal to k 1 e naught s divided by k minus of 1 plus k 2 plus k 1 s. Now, our enzyme reaction is what E plus S equal to E S, and then E s giving you products plus enzyme. Therefore, the rate at which rate of formation of product is k 2 times e s. This is the rate at which product is found. So, we have got e s here. So, I can get rate of formation of products.

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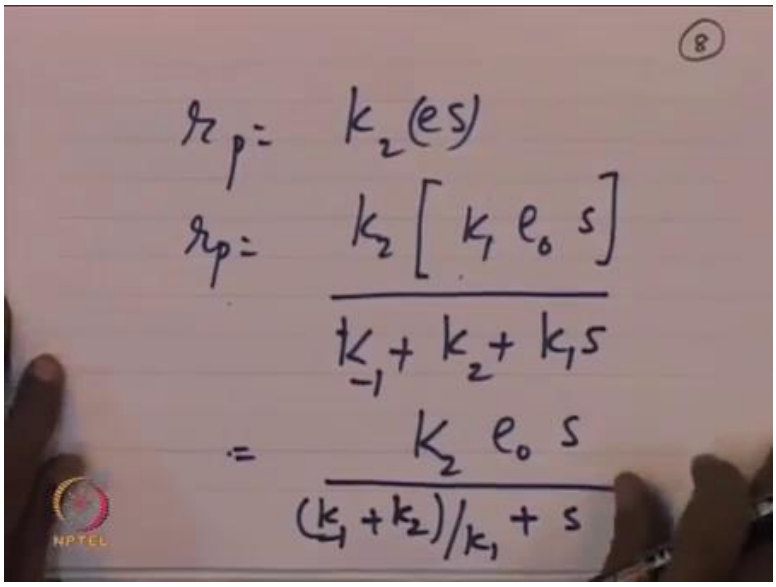

Let me write here r p is k 2 times e s therefore, r p equal to k 2 and e s is ((Refer Time:

09:38)) this one k 1 e naught I will write it again. So, it is k 1 e naught s divided by k minus 1 plus k 2 plus k 1 s. So, I can simplify this little k 2 e naught s divided by k minus 1 plus k 2 divided by k 1 plus s. So, all I have done is I have just divided numerator in denominator by k 1. So, I have got s here k minus 1 plus k 2 divided by k 1. Let just recall what is k minus 1.

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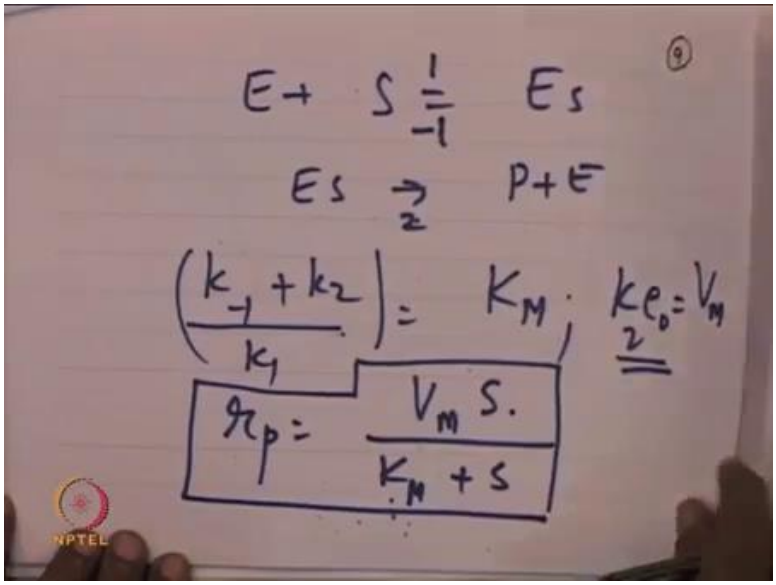

Let me, quickly write it again E plus S equal to E S. This is 1 and this is minus 1. So, E S going to products plus enzyme this is 2. Therefore, k minus 1 plus k 2 divided by k 1 this term is often called as K M. This is the nomenclature and enzyme let us them k 2 e naught is often called as V M. Therefore, r p is denoted like this. This is the way in which you will find in literature along the world. The enzyme reaction kinetics is represented like this.

But, V M is k 2 e naught and then K M is k minus 1 plus k 2, k minus 1 plus k 2, you can see here k minus 1 plus k 2.

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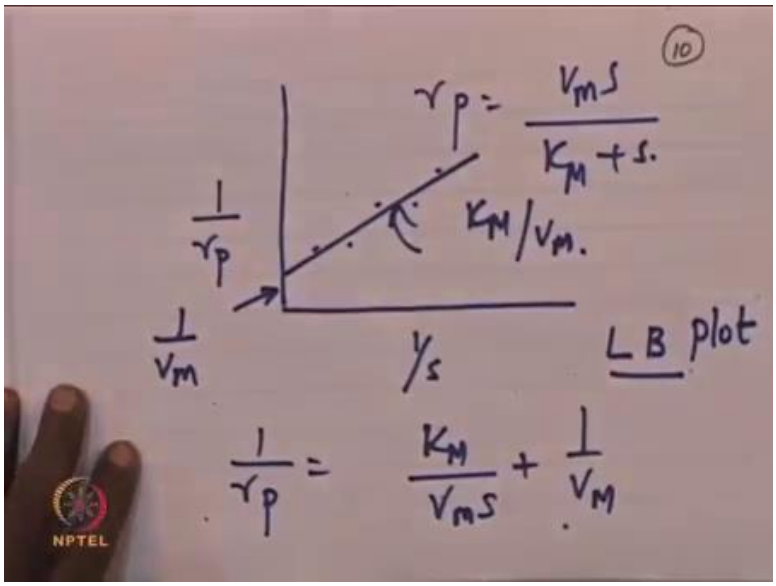

Now, if you want to find out the parameters of the enzyme reactions typically, what people do is this. Make a plot, so I will try this again r p equal to V M s divided by K M plus s. So, when we do an inverse transformation 1 by r p equal to K M by V M s plus 1 by V M. This is of course, we have done this many times with the past. So, this is often called as line be the birth plot L B plot.

This is the way in which it is describe so you get straight line like this, you have points like this. So, this intercept is 1 by V M. And this slope is K M by V M. On other words in enzyme reaction data, if you plot in this form 1 by r p was 1 by s slope and intercept gives you all the parameters.

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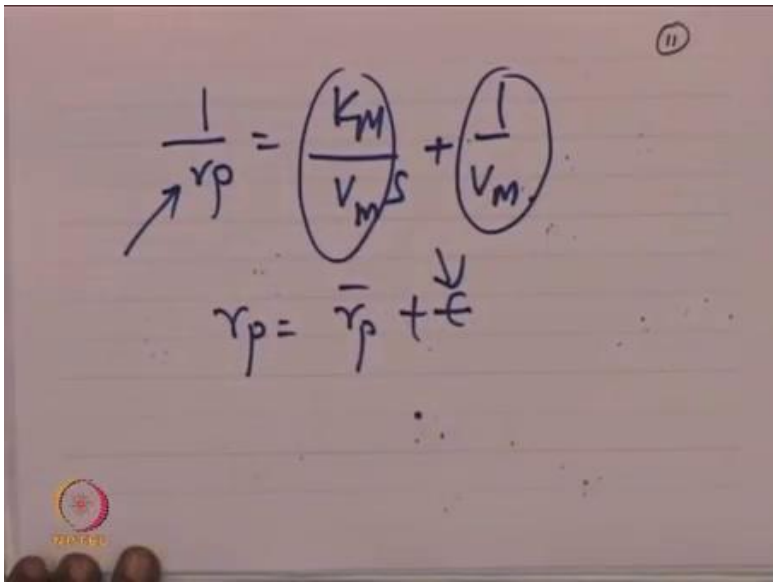

Now, what is important to recognize here is that. Whenever, we do an inverse transformation. let me write this once again 1 by r p equal to K M by V M s divided by 1 by V M. So, when we do an inverse transformation, what happens is that we do measurements in r p. Therefore, r p when we measure, that is some standard values of r p bar plus an error. When we do an inverse transformation, this error is no longer have been this same distribution on same normal kind of distribution.

So, inverse transformation have this problem that the error distribution get affected. Therefore, estimation of parameters by doing an inverse transformation by itself is not a good thing. So, what is generally done is that, these K M by V M that comes from this is taken has the initialize estimate to do an non-linear search. Which means ((Refer Time: 13:23)), you should use this values of K M and V M that comes out of this linearization and it should be a non-linear search.

What is meant by non-linear search? That means, these parameters ((Refer Time: 13:34)) and occurring the non-linear way. We should a non-linear recursion to find out V M and K M, using this as the initial approximation. So, K M and V M coming out of linear is estimation to be used only as an initial approximation for a non-linear search. So, the non-linear search is the best rate it determining the parameters. And you should not give too much confidence to the K M and V M, that comes out of the linear estimations.

L B means, this way of representing the enzyme data is called as line weaver Burk plot.

This is the name, this comes out of those people who did this meant the many years ago. Line weaver Burk, line weaver I can little line weaver Burk.

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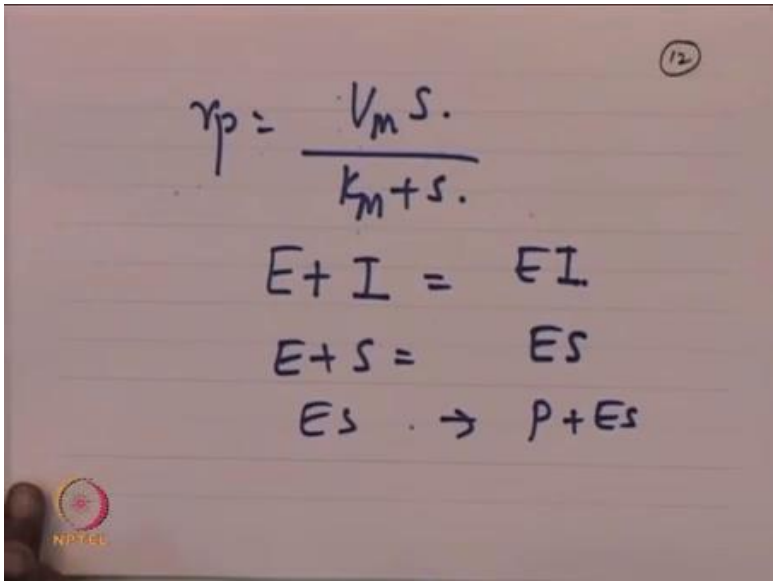

Now, in form to go kinetics as you all know that r p equal to V M s by K M plus s. So, what is normally done is that, we want to control certain processes in a say a medicine that we all take. The object is to administer this medicine through the particular path way is stopped or in it in enhanced activated whatever. So, this is lot of material that is available, in which this enzyme you can inhibit this enzyme by putting in appropriate chemical.

So, when you inhibit this enzyme, what happens is that, this the enzyme goes and then block some active sites. So, when you put your substrate, this substrates compete for the same active site correct. So, that you have to reaction now takes place, because only as part of the active site is now available to the enzyme. So, you can have what is called thus competitive inhibition, in which the enzyme goes and blocks the active site.

Now, we have talk to about enzymes, have talk to about what enzymes are we said enzymes are proteins. We also talk to about a very simple form of enzyme kinetics, if you call us Mycale is might be the kinetics. And we said, will talk me about Mycale is meant in kinetics.

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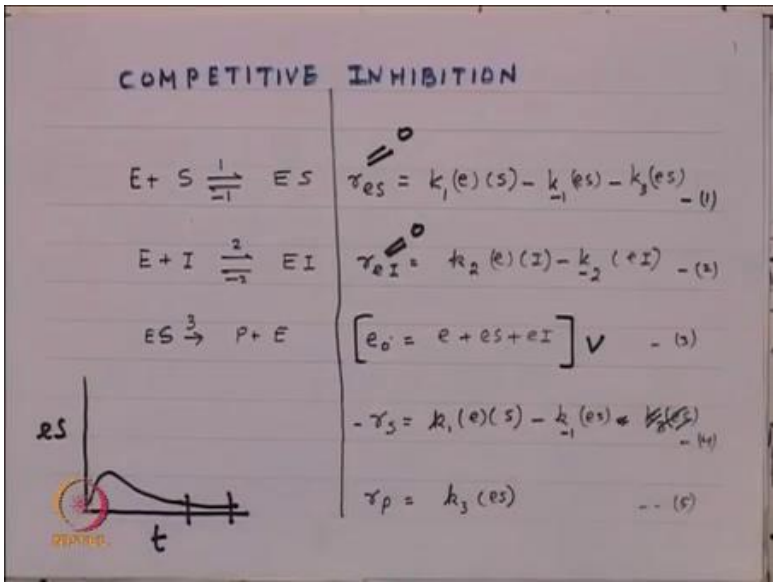

That the basic formulation of Mycale is meant kinetics is that, enzyme reacts with substrate forms complex substrate to form an E S complex. And E S complex decomposes to give you product enzyme. This is the basic of formulation of Mycale is kinetic, meant it kinetics. Now, over the E S people we have understood, that enzymes do get inhibiter you to variety of reasons. And so and in form of co kinetics in medical treatments in all that. We often look at use the inhibiters to manage certain reactions.

Therefore, if I is the inhibiter, which complex as with the enzyme. So, that it forms that E I complex. On other words, what we are saying is that you could have a situation in an enzyme process, that substrate complex is that enzyme to form the enzyme substrate complex. And inhibiter complex is the enzyme to form inhibiter enzyme complex. So, that the active sites on the enzyme is partly consume by the inhibiter. So, that the amount of sites available for substrate is less this could happen.

So, that the E S, which is decomposing to form product that enzyme. That is less of E S complex that is available. And to that extent that reaction rates are affective. Now, the context is something like this. That in enzyme literature is tremendous amount of material on how an inhibiter performs on enzyme. So, the use of inhibiter for managing enzyme reactions are very common and that is one of the reason, if I looking at this kind of smart this.

Now, I mean the basic procedure for understanding enzyme kinetics, you already done.

So, you write the complex is r e s complex r e a complex. What is r e s complex that means, the rate at which the enzyme substrate complex is found. We can see here, E S is found from reaction one. So, it is k 1 e times s you can see here. E S is found reaction k 1 e times s. It is consumed in reaction minus 1 therefore, the minus k minus 1 e s. At the same time, you find that E S is consumed in reaction 3 there was minus k 3 e s.

On other words, the rate of formation is e s we have take into account the reaction in which e s is found at reaction is e s consumed. So, all that is written here that is r equation 1. Now, if you look at equation this reaction E plus I equal to E I, you notice that r e I complex it is found from k 2, k 2, k 2 e times I is the formation of E I complex. And minus of k minus 2 e I is the consumption E I complex, that is reaction 2.

Now, you also know that whatever enzyme we start with then that enzyme is partly free, partly complex by the e s complex partly complex with e I complex. So, that this is the material balance for enzyme. Of course, we can put in the multiply throughout by v to sure that is the total amount of materials that are enzyme the equipment if it to the batch the different and so on.

Now, the rate at which substrate s, minus of r s. When that means, rate at which substrate s is consumed, s is consumed in what are the reaction in which s is consume. That reaction which enzymes is consumed k 1 e times s. You can see here, k 1 e times s is consumed at reaction k 1 e times s. Now, s is also consumed in which the other reaction s is consumed. k 1 e s reaction 1 it is consumed. And then the reaction minus 1 it is formed, s is formed.

So, k 1 e times s, minus of k minus 1 e s is the rate of formation of r s come formation of substrate. The rate formation of product is simply what k 3 times e s that is enough function of product. Now, something that it like you do for the recognize I am not derived write here so elementary was thought is not the essential. That when you put the e s complex equal to 0. Let means, we say that this is stationary. This is equal to 0, this is equal to 0. When we say, that r e s is stationary.

What is meant by r e s is stationary, when you say r e s is stationary, what is meant is that, if you make a plot of e s versus time. e s is not a go through a like this versus, what we are saying is that we are actually looking at the process, during this in this phase. Where, e s is not change. Essentially, what we are saying the e s is not change, e s is

constant. That is the meaning of r e s equal to 0. The rate at which e s is change in this not important it is called quasis studied state approximation.

Now, when you put r e s is equal to 0, what is that that reaction 1, this equation 1 k e s plus k minus of all that this is 0. Now, if we look at equation 1, reaction 3, equation 5 and 4. You realize that under the quasis steady state approximation r p equal to minus r s. Or rate of formation of product is equal to the rate of consumption of substrate. And that make sense also.

That is the, but what happens here is that, the presence of E I inhibiter as slightly introduces the element of reaction rate decreases, which we will at come to shortly. So, what we are saying now, what we are saying now is that r e s equal to 0, r e I equal to 0. And then the material balance for the enzyme for given by the equation 3. And under the quasis studied state approximation r s, r p equal to minus of r s, which you can derived it is such not the problem. With these, we can now go on and determine what is the kinetics, for the case of competitive equation.

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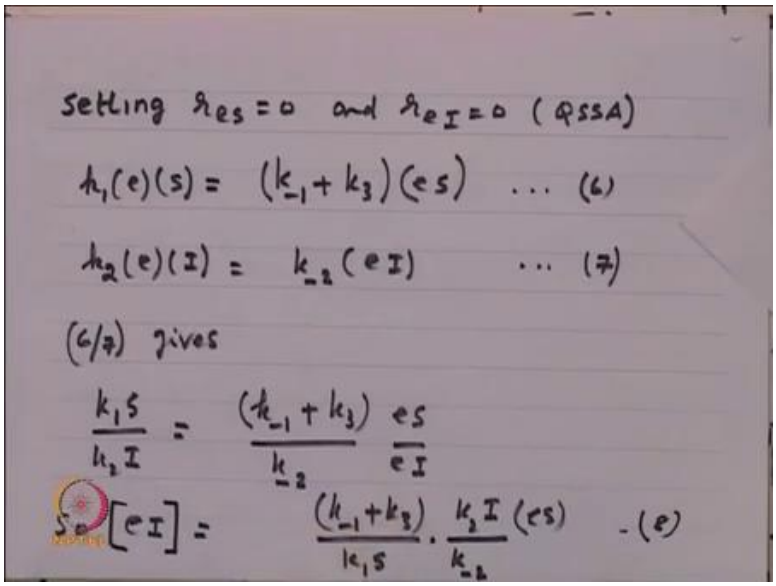

So, what I have done is that adjust we written equation 1, equation 1 ((Refer Time: 22:25)) I have written like this, I take other side. And similarly, I have written equation 2 slightly differently. So, the idea of writing equation 1 and 2, what I have done in that I have taken this put as 0. I have taken it one side I have taken this one side. So, it sort of the use at advantage in terms simplification it. So, when I write it a this form equation 1

and 2 as 6 and 7. I can divide 6 by 7 e cancels of see.

If I divide the e cancels of so that I get directly in a from here the e I complex. That means, from equation 6 and 7 I am able to tell. Then what is the amount of e I complex found. So, you can see very clearly that e I equal to k minus of 1 by k t k minus of 2. This is equation I can call the equation 8 ((Refer Time: 23:20)). So, what we have done is that equation using equation is 1 and 2 ((Refer Time: 23:26)).

And rearranging we have eliminated e I got at expression for e I complex. That means, e I now depends on k minus 1 k 3, k 1 s, k 2 can and I e s and so on.

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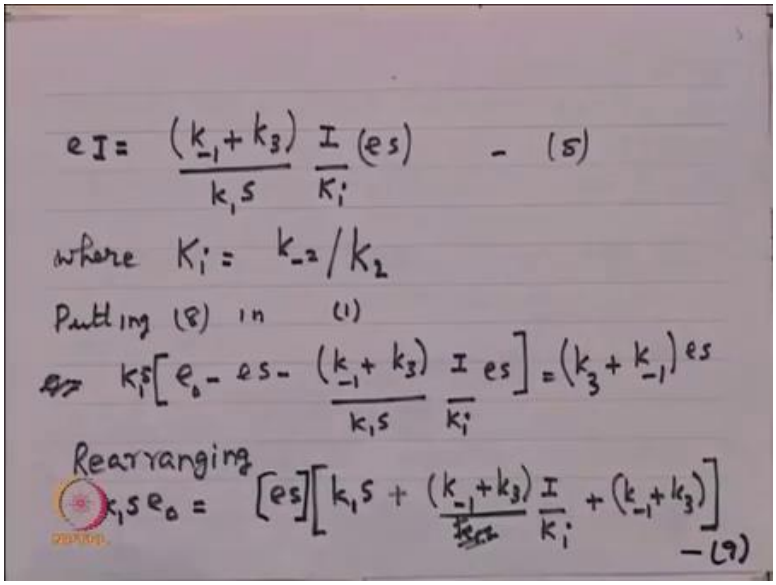

Now, so the advantage enzymes is that, so I have just what I have done here is that, have the e I complex I have just got the e I complex here the equation 8. Then I have substituted for e I ((Refer Time: 23:55)) from here. So, what I have done here is that, I have substituted for e I from equation 8. So, what do we get, what do we get is that I substituted for e I here and then simplify. So, what do we get, we get ((Refer Time: 24:13)) let me go through this once second just for all.

So, what do we have, what we have here is that r e s equal to 0. k 1 e s k minus 1 e s k 3 e s. Now, this e we can eliminate from equation 3. What is e equal to e naught minus of e s minus of e I. So, what I have done is the following. From equation 6 and 7 just know we talk to about the equation 6 and 7. So, from equation 6 and 7 we found out what is the

e I. Now, we know e I, now if you go to this equation 3 ((Refer Time: 24:52)).

Now, you want to element this e here and equation 1 can be written as e naught minus of e s minus of e I. What we have done is that, we have substituted in equation 1. The replace is e from equation 3 and then what you substituted for e I we already calculated and the whole thing simplifies. Simplifies very nice it, simplifies something like this. It simplify is as that it simplifies like this. So, that what we get is that, the e s that means, you can see here e s appears the everywhere e s appears.

So, we can then to determine what we have done is that, we have determine, what is the amount of e s complex that exist in the process in the presence of inhibiter. So, you can see here, this equation 9 I call this equation 9. We find that equation 9 tells us quantitatively. How the quantities v s complex is related to system parameters. So, that is has be done. So, we are able to determine from equation 1, 2 up to 9.

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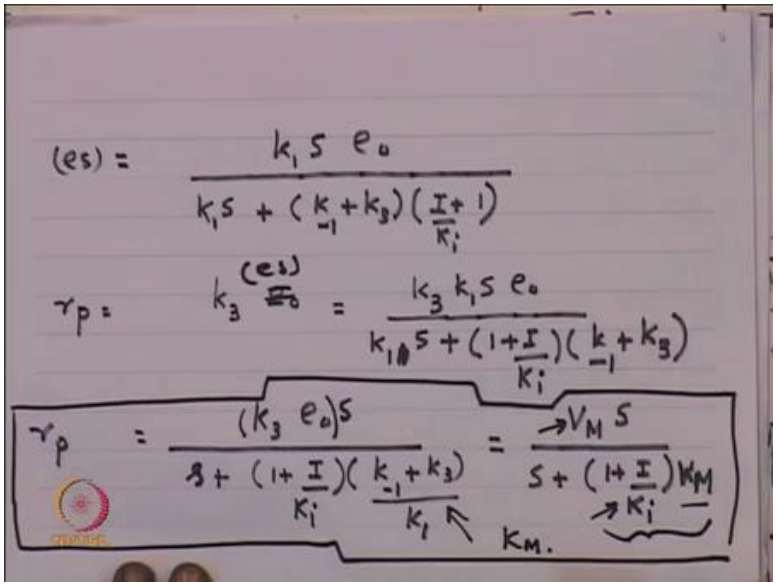

That e s complex, in the case of competitive this kind of competitive inhibition is given by this expression. Notice here, that in this expression it involves i the co amount of inhibiter your added. And also involves k i the ionization constant for the inhibiter. How did define ionization constant? We ((Refer Time: 26:15)) define ionization constant k minus 2 by k 2. What is k minus 2 1 k 2 ((Refer Time: 26:22)), this is k minus 2, this is the ionization of E I is called minus 2. And that reaction e plus I is called k 2.

So, ionization constant is k minus 2 by k 2. And with that simplification with that formulation with that, define that the e s complex can be given by this expression. Now, this is from elementary algebra involved. So, I could up for derived within, but I thought it is not necessary. So, elementary that ideal thing is important spend time on this. So, I have got the e s complex here. And then r p is defined as k 3 times. What is r p rate of formation k is it given as k 3 times e s.

So, e s is given by this. Therefore, the rate at which the enzyme reaction, which occur given by this expression. What we have got here is the rate of formation of product is given by this expression. Now, what we have I got here this k 3 e naught is represented as V M. And then, this term k 1, k minus 1 by k 3 by k 1 is denoted as K M, this is K M, this is denoted as K M. So, the rate of formation of product is given as V M as divided by s plus K M multiplied by a term, which is determined by the inhibiter.

Let us, ((Refer Time: 27:51)) just go through this once second. Just for the sake of understanding the basic approach of determine the chemical kinetics. Let me do this once again. Just to emphasis to you this is vary the elementary does nothing much the very elementary algebra is involved, at did not do it, because it is not necessarily. What it we do, we said ((Refer Time: 28:13)) this is the case of competitive inhibition. What is competitive inhibition?

Competitive inhibition is an instance, where the inhibiter attaches itself to the enzyme, so blocking some of the active sites. Because, it block some other active sites the amount of e s complex that is formed is less than in the case, where there is no inhibiter. Now, what I have we done, we have written the rate of formation of e s complex e I complex and the material balance for the enzyme. We also write the rate of formation substrate and rate of formation for the block.

We said, under the quasis steady state approximation where e s is 0. We can show I am not shown this very elementary that r p equal to minus of r s. On other words, whatever product, whatever substrate is consumed that may products is formed under the quasis studied state approximation. You also said at this approximation is valid, if e naught by s naught is small that means, the amount of enzyme to the amount of substrate is small. Now, having set this, we said that we can now by looking at reactions 2 and 3.

We can makes some rearrangements and find out what is the amount of e I complex that

is formed. When we said, that the amount of e I complex that is formed can be shown to be equal to equation 8 ((Refer Time: 29:35)). It is something vary elementary. Now, when we said that it is now possible to the eliminate ((Refer Time: 29:43)) this equation 1, we can be replaced as e naught minus of e s minus of e I. Therefore, you can substituted for e is replaced by e naught minus of e s minus of e I.

Then both e I can be given in terms of e s ((Refer Time: 29:55)). Therefore, we can get and equation for e s that is what we taken. It said, now e s can be said to be equal to v calculate at that e s can be given by this representation. Now, we know that the rate of formation of product is k 3 times e s. Therefore, we got r p if rate of formation of product under the conditions of sub competitive inhibition is given by this expression. Notice here, the form of this is very, very similar to the Mycale mental.

Mycale mental is what? Mycale is mental equation is r p equal to V M s by s plus K M. Instead here, instead of K M it did the multiplied by term 1 plus I by k i. On other words, what happens here is that in competitive inhibition. The K M value or absorb value of K M is higher than you would have got in when you had no inhibition. So, that is the only point that you should the apply essentially, what it means under competitive inhibition that K M becomes higher than in the previous case with there is no competitive inhibition.

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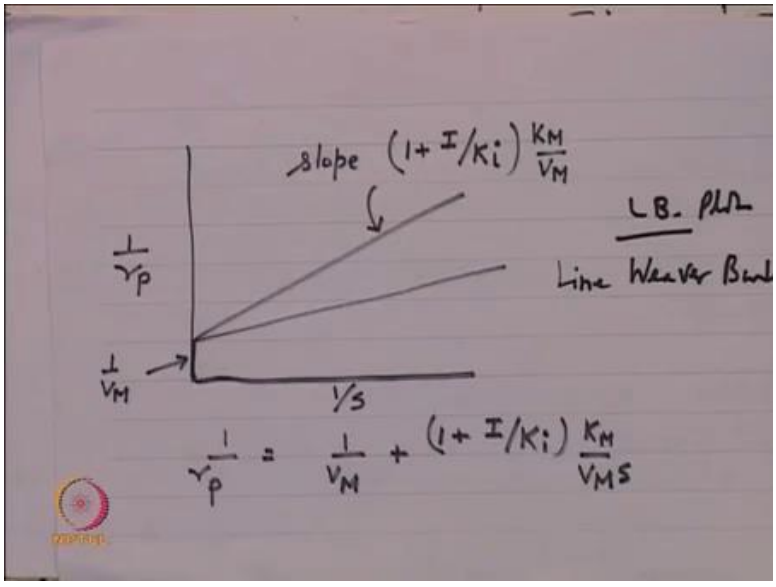

We can understand this is another way, which is the way in which is available the

literature this is called as the line weaver Burk plot. What is called this 1 by r p were it is called line weaver Burk plot also called as L B plotted. In L B plot, if you plot 1 by r p versus 1 by s will be out r 1 by r p that is what I have done here. In notice here that, it is called the same intercept as before, but this slope is higher.

Showing that, the K M value that we will get under competitive inhibition is higher than what you would get, when there is in no competitive inhibition.

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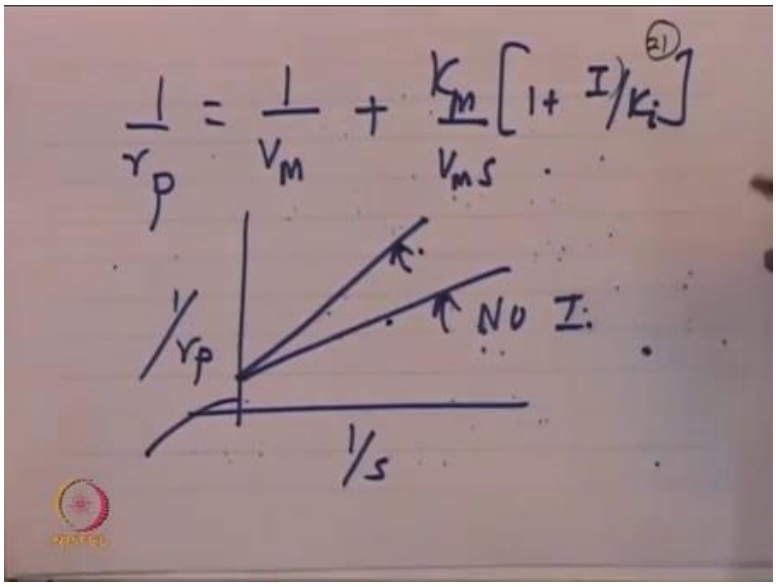

So, when we make a plot of 1 by r p versus 1 by s, you get a higher previously you had this, then there was in no inhibiter, no inhibiter. When there is inhibiter the slope becomes K M by V M times this whole thing. Showing all that is being said, then there is a competitive inhibition the K M by in the line weaver Burk plot, you will find that the slopes are higher than what we go before. And that that represents the effect of the inhibiter.

Suppose, you have to find out these numbers in an experiment, what you will do is that. You will do an experiment without inhibiter. You will do an experiment with inhibiter. So, that by some these two you can find out V M, this is V M. This gives you K M and this gives you k I. When we do our experiment with inhibiter without inhibiter, without inhibiter you would k M and V M. When you put the inhibiter, since you know the concentration inhibiter you will know k I.

So, in it about two experiment to able to get the values of K M, V M and k I Now, there are many types of inhibitions that of described in a literature.

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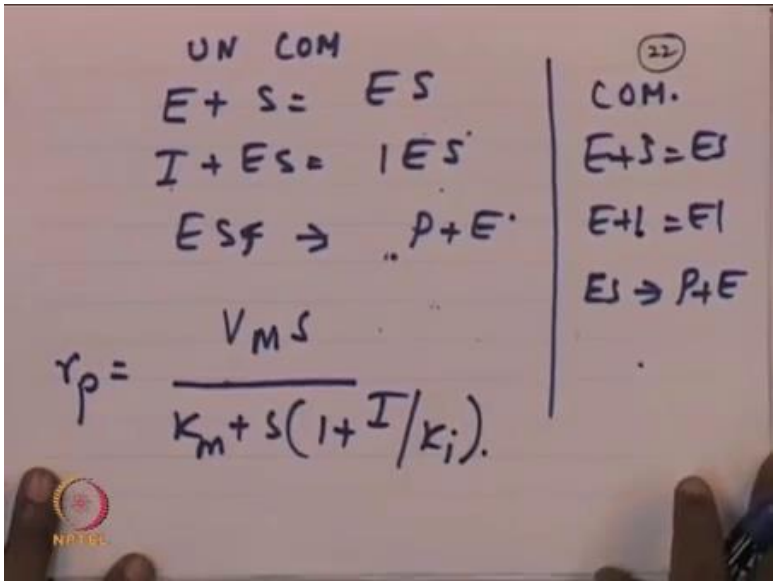

I will quickly set out the other types of inhibition E S, I plus E S we said I E S. E S giving you product plus enzyme. Now, please compare this with this is E plus S is E S, E plus I is E I and then E S giving you product. Now, this is what is called as this is competitive. This is called uncompetitive. What is the difference between these two? In competitive inhibition the enzyme active sites this inhibiter goes and sits. But, in uncompetitive inhibition the inhibiter sits on the E S complex.

You can see here now. So, it depending upon the way it actually, you can show I will write only the final form. Because, it mean in just form this how will get.

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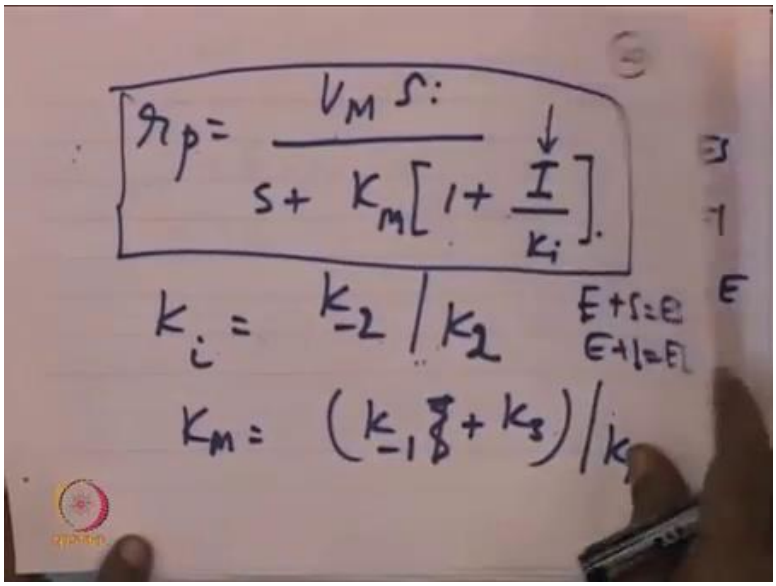

Please notice here in the competitive inhibition the K M gets multiplied by this term 1 plus I by k i. In uncompetitive the inhibition the s term in that denominator gets multiplied by the term. So, this is the, you can by looking at so in the line weaver Burk plot.

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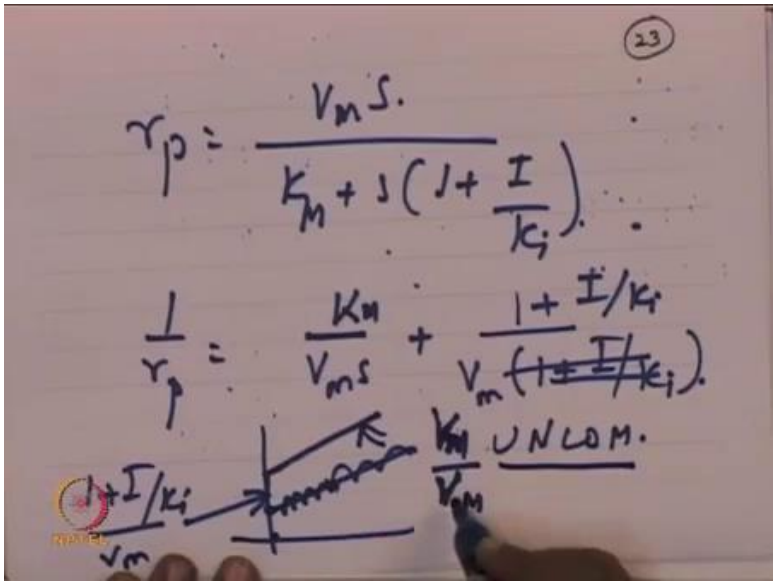

If you make a plot of this r p equal to V M S divided by K M plus s multiplied by 1 plus i by k i. So, when we do 1 by r p, becomes 1 by what it is K M by V M s plus what 1 by V M multiplied by 1 plus I by k i. So, in this plot here without so you have slope is the

same correct. So, intercept is higher. So, I should ((Refer Time: 35:27)) plot it here I think.

Student: ((Refer Time: 35:29))

Is it ok now,, so you have a higher intercept, the intercept is previously it was now it is intercept becomes larger. It is a larger intercept for 1 plus i by k i times V M. So, this is the intercept. So, in this case we have a larger intercept same slope while in the previous case we had same intercept to higher slope. See, this is competitive correct this is competitive yes or no, this is competitive inhibition where the slope is competitive inhibition you have same intercept, but higher slope.

Now, in uncompetitive inhibition, where are we?

Student: ((Refer Time: 36:25))

This is the uncompetitive inhibition here, you have same slope. But, higher intercept. So, same slope, slope is K M by V M, this is K M. So, it this uncompetitive slope is the same, but intercept is higher. Now, there is one more form which is also seen in the literature let me quickly write this.

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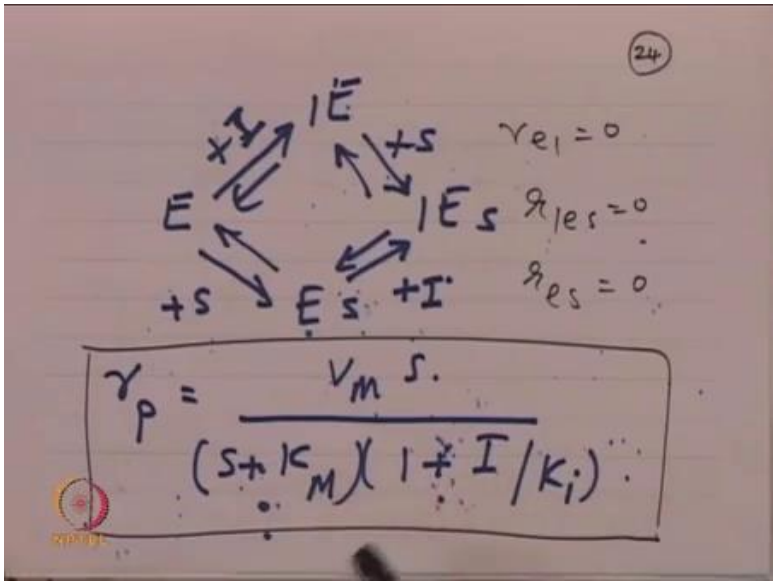

So, here is an instance, here is an instance where enzyme combines which substrate. So, combines of I to form I E complex combines with s to form I E S complex can combines

with s to form E S complex combine the I E S complex. So, here it is an instance, where you have E plus I giving I E competitive, then I E S uncompetitive in here it the other way E S and then. So, it is a combination evolve this. So, when you go through this sentence put this quasis studied state approximation.

You have r p comes something like this V M, I want derive this it is a little missing it can do it yourself. So, when you so you can see here, V M s in that denominator now. the I plus k i multiplies both s as well as k M.

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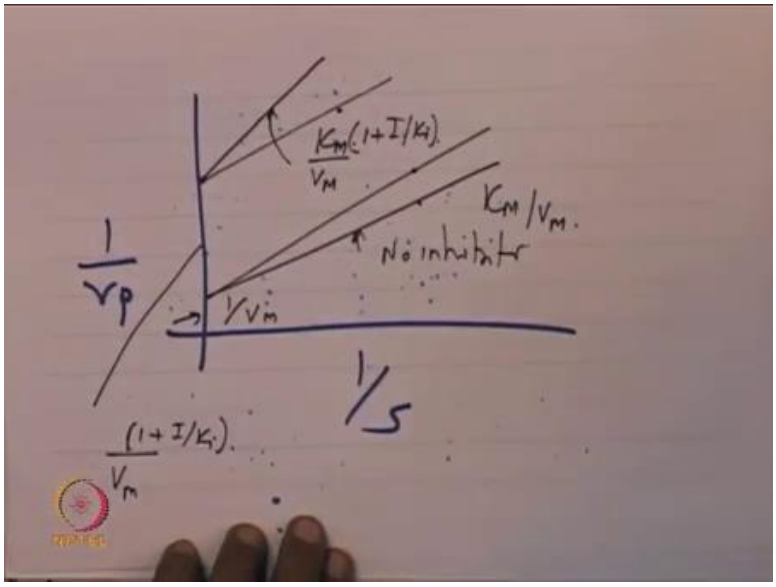

So, when we do this line weaver Burk plot it may plot it here, 1 by s were 1 by r p. So, you have this is no inhibiter. So, this is V M, 1 by V M. And this is K M by V M. Now, when you have this ((Refer Time: 38:57)) kind of inhibition you will get a slope am drawing it like this. So, this is a slope here is K M by V M multiplied by 1 plus I by k i. And this intercept is 1 by V M multiplied by 1 plus I by k i. So, we have for 1, 2, 3, 4 types of a enzyme modules will do one by one.

This is an instance here when there is no inhibiter. This is an instance, where there is an inhibiter competitive inhibition. Here this uncompetitive inhibition. So, the nomenclature is a little I am not convincing, but what is important to recognize is that this is competitive. Is here r e i equal to 0, r i e s equal to 0. And then r e s equal to 0. These are the assumption and that is 1 more assumption, which is implied here, which is done in the enzyme literature.

That is the ionization constant for inhibiter attaching to I E complex and attaching to I E S complex as long as inhibiter attaching that in a ionization constant does not change. Now, this is assumption is made that means, I is attaching to E I whether it attaching it E S or on E I it does not matter. Because, the sites of the set. Let us, how those they assume that kind of inhibition constants. So, you can do this yourself it is not very complicated.

So, what I want to summarize here is that to form of this function, when you do it on the line weaver Burk plot you get us a straight line from where you can get your inhibition constants. So, only thing is that, this use should be use it as the initial approximation for an non-linear search. That is up. Do not use this directly for your estimation, because they have lot of errors associated with it. Now, the important thing that we should know is,

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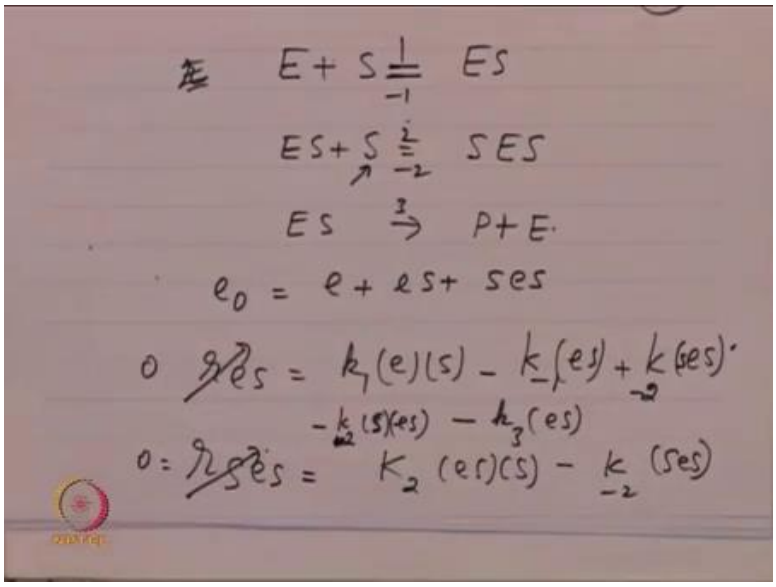

What happens in enzymes systems is the following typically, what happens is you get this E S. And now frequently this substrate goes and binds. So, that you are E S complex gives you P plus E. Now, this is an instance of your substrate itself blocking the reactive sites. So, that the rate at which reaction now takes place is affected by the fact that s is blocking the S E S complex. Now, this is not uncommon see for example, what happens is that in commercial process, where we tent to use the large quantity of substrate.

Because, we want to process in large quantities, then that is when this seems to happen that s goes in blocks the enzyme. Now, we want to quickly derive this, because it gives

some interesting relationships. So, let me write this e naught equal to e plus e s plus s e s. Then you have r e s equal to k 1 e times s minus of k minus 1 e s plus k plus minus 2 k minus 2 e s. Please tell me, this is let me put all the nomenclature 1 minus 1, 2 minus 2 and 3, so e s k 1 e s minus k 3 e s.

That this correct, k 1 e s, k minus 1 e s, k minus 2, I have to write that also minus k 2, that I have to write. is it ok now. So, then r s e s equal to k 2 e s times s minus of k minus 2 s e s.

Student: ((Refer Time: 43:35))

K 1 just let me go through k 1, k minus 1, k in to s e s this is ok. Now, this goes to 0, this goes to 0. So, when you set up all the equations this is not all this little bit miss here I will write the final form looks like this.

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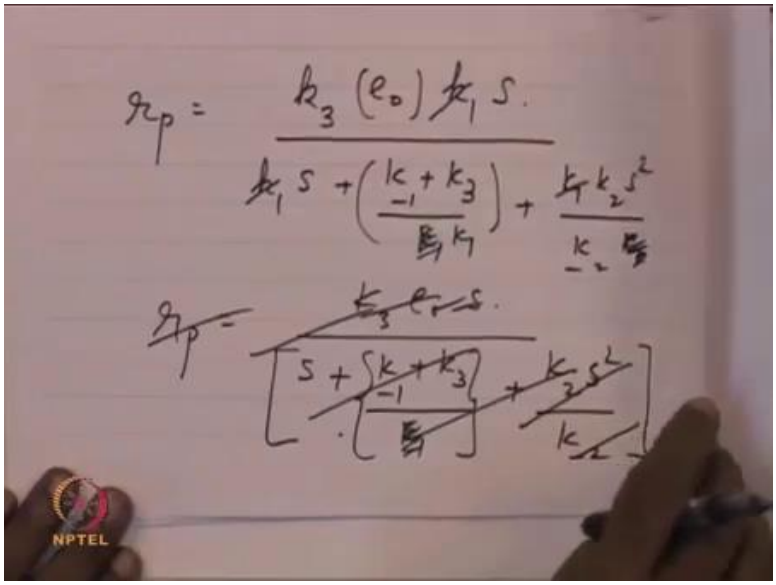

r p when you calculate, it looks like k 3 e naught k 1 s divided by k 1 s plus k minus 1 plus k 3 divided by k 1. And then k 1 k 2 s squared divided by k minus 2 k 1 simplifies finally the simplifies as r p equal to k 3 e naught s divided by s plus k minus 1 plus k 3 divided by k 1. And then k 2 s squared divided by k minus 2. This it is sort of look like this. And in the literature this term comes s squared.

Student: ((Refer Time: 44:58))

I am not it is not there. Because, I have put k 1 at the top. Now, when I divide throughout by k 1. I knock out is this, knock out this then I will put k 1 here. So, this is what I will remove this. This how it is done in the literature, so this term is called. So, it becomes k 2 by k minus 2. So, now let me write it in the form in which available in the literature

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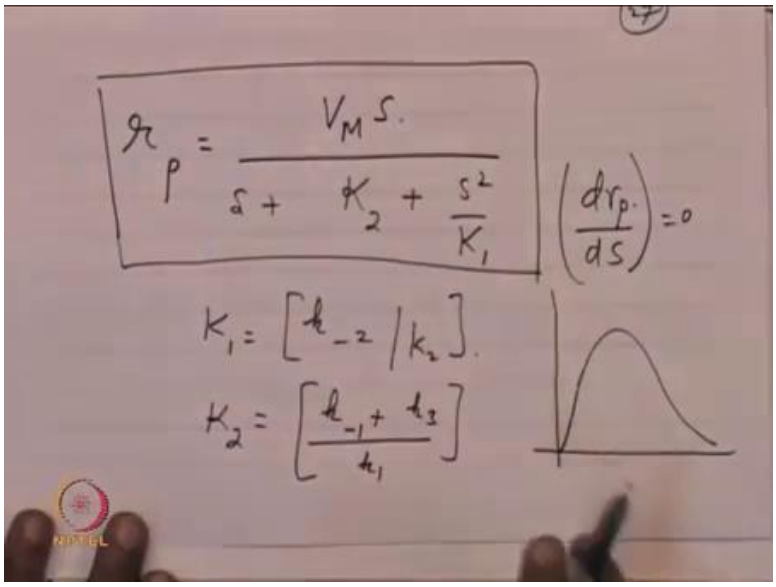

R p is available like this V M s divided by s plus k 2 plus s squared by k 1. Why k 1 is k minus what is k 1, k minus 2 by small k 2 cut. So, ((Refer Time: 45:57)) and this term k minus 1 ok, k 1 is ok. And then other 1 is k 2 is k minus 1 plus k 3 divided by k 1. This how the available in the literature. Now, the reason why I am doing this is the following. This is an instance of the rate function going throughout maxima.

You will find frequently in the enzyme literature, that the rate function actually, goes through a maxima. And this is how it is explain in the literature the maxima is because the plus substrate inhibits the enzyme complex. And this ((Refer Time: 46:49)) kind of model applies and that is why, this kind of functionality see. Now, if I ask you know, what is the if it do d r p by d s equal to 0. Can you quickly do this for me, on this r p V M s by s plus k 2, s squared by k 1, what is d r p by d s?

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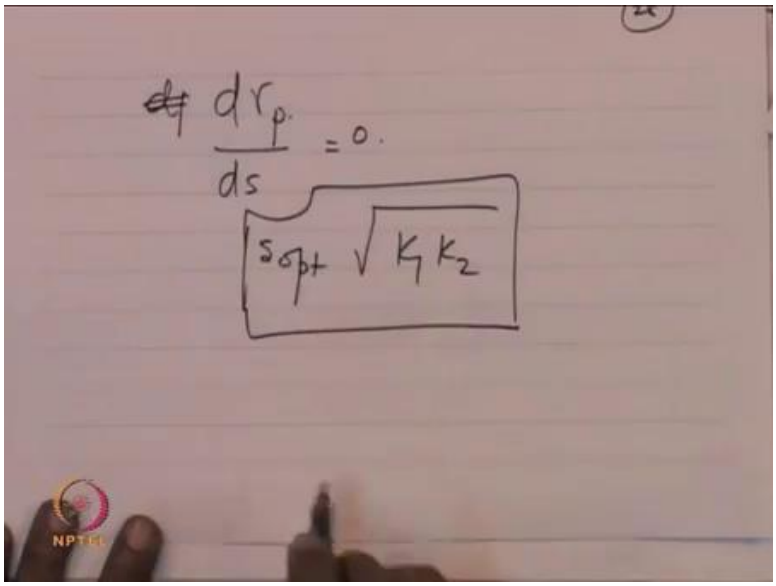

If you put d r p by d s equal to 0. I get this happens only 1 s optimum, becomes equal to k 1. This is an interesting result in enzyme literature, ((Refer Time: 47:32)) that this whenever we have substrate inhibition, that the highest rate you obtain when the value of s substrate that we use e square root of k 1 k 2. This is an interesting result. But, k 1 and k 2 are the 2 into constants of the model. And square root of k 1, k 2 is the best value at which we should operator.

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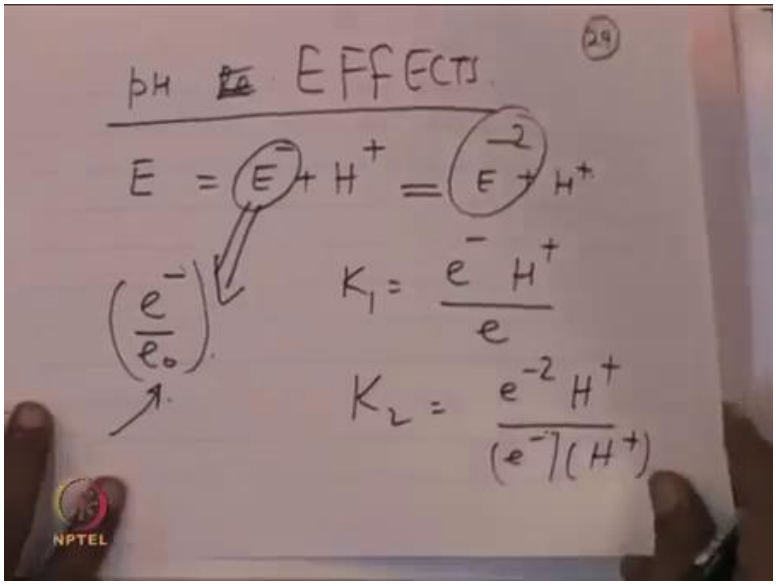

See, the reason for doing this is the following. So, what happens is an enzyme terms to

ionize like this. Please recognize this using by this particularly model ((Refer Time: 48:17)) of enzyme reaction as become very interesting is, because enzyme tends to ionize in this form. And the e minus is the active form of the enzyme. On other words, if you put in so much of enzyme it is this fraction that seems to be active as for as the enzyme reaction is concerned.

Now, let me write what is k 1? k 1 is e minus H plus divided by e. And k 2 this first reaction second reaction is e minus 2 H plus divided by e minus 1 and H plus. So, what is k 1 and what is k 2. k 1 and k 2 are the ionization constant first ionization, second ionization of the enzyme. See, enzymes of the active sites of the ionize and it is this which seems to be the active form of the enzyme. And, but it ionizes further this an inactive form.

So, what is the active form of the enzyme, if you want calculate using the values of k 1 and k 2. So, you want to find out, what is e naught minus by e naught. Let me, what is e minus by e naught. Let me, quickly calculate that.

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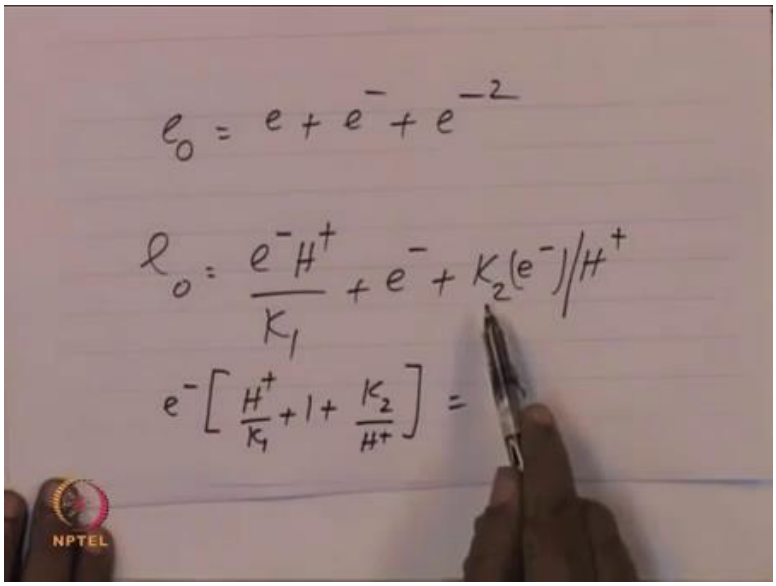

So, total enzyme e naught equal to e plus e minus plus e minus 2. So, e naught equal to from here, what is e equal to e minus H plus divided by k 1 that is the first term ((Refer Time: 49:53)), e equal to e minus H plus by k 1. The second term I retained like that e minus. Third term ((Refer Time: 50:02)) e minus 2 is what k 2 e minus H is correct what I have written.

Student: ((Refer Time: 50:07))

So, it will be squared here.

Student: ((Refer Time: 50:11))

So, what are become e minus 2, e minus 2 becomes k 2 e minus, e minus h plus. So, you have e minus within brackets H plus by k 1 plus 1 plus k 2 by H plus equal to e naught, e H plus by k 1 plus 1 k 2 by H plus equal to e naught.

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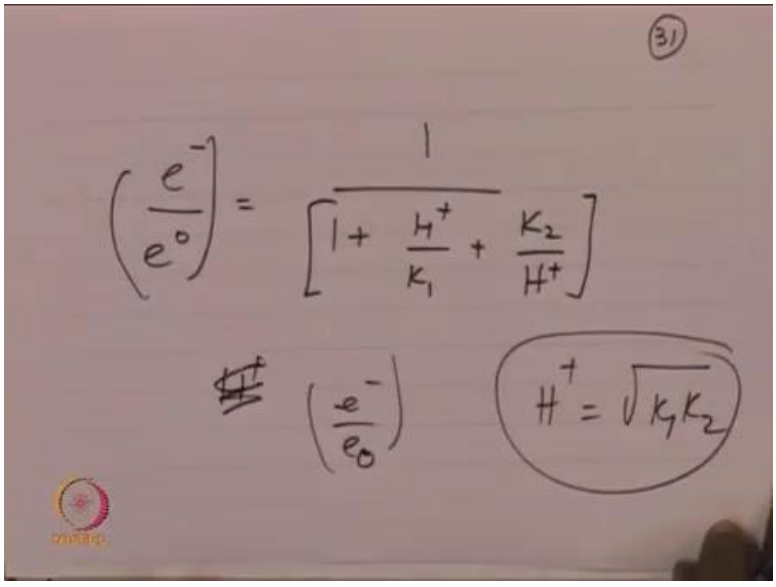

E minus by e naught equal to 1 divided by 1 plus H plus by k 1 plus k 2 by H plus. Let us, just see the form in which we had the previous. See, if we just look at this ((Refer Time: 51:11)). If I just divide by so I just divide by s here. So, you can see here 1 plus that forms of identical H plus by k 1. You can see at s by k 1. And then, k 2 by H plus k 2 by s. So, the form of the substrate inhibition that we said for enzyme kinetics.

And the way in which the hydrogen ion affects the e minus on the active form the enzyme the form in identical. Showing that h plus actually serves as the way of inhibiting the enzyme kinetics. On other words, what we are saying is that in enzyme kinetics choice of hydrogen ion concentration is crucial. So, if we choosing properly then we get very high activity. If we do not, you do not good activity. And what is the best choice p H. What is the highest value of e minus by e plus.

You will find that the optimal e minus by e naught. You will get when we choose H plus equal to root of k 1, k 2. So, this is the best p H of operation of an enzyme reaction is the root of k 1 plus k 2. So, this is the most important message that we want to carry home from the enzyme literature. Now, with this backround in enzymes. So, what just we quickly summarize.

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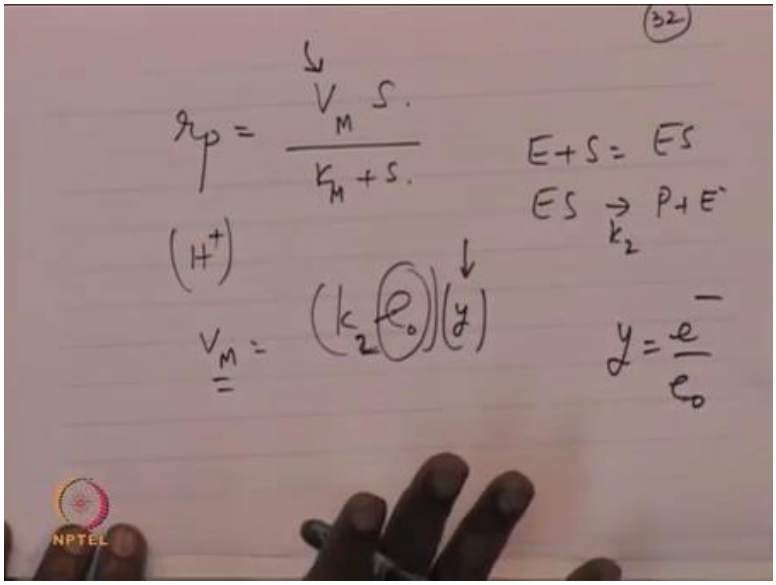

What we are saying is that, the rate of enzyme reaction is this. Now, we are saying that choice of H plus gives you a proper reaction rate. And then, the effect of H plus can be accounted for by a form ((Refer Time: 53:06)) of this types, where the active form of the enzyme is given by this relationship. So, that if we choose express thus you good very good reaction rates. Now, what is V M? We said V M is k 2 times e naught. That is what is a V M. So, our model is E plus S is E S.

And then E S giving you product plus enzyme. And this is k 2. And V M is k 2 times e naught, that also we said. Now, this e naught is not what is the active, what is the active is travois only this. So, to that the extent effect of p H 1 come into play. That means, in which in that e naught is the, at the working for us, but what works for you is only e minus. And therefore, the effect of e minus by e naught will actually appear here. So, in here it says k 2 times e naught. But, actually it is k 2 times, e minus e naught.

So, e minus that effect will come in the reaction. On other words, V M that you will absorb will be slightly less. Because, of the fact that ((Refer time: 54:13)) the effect of p

H on the enzyme, because of this relationship. Once second, what we are saying is the rate of enzyme reaction is V M must be k plus s. So, we find in many cases the choice of p H is crucial to the rate of chemical reaction.

What is the best choice, best choice ((Refer time: 54:35)) comes from understanding that e minus, which is the active form of the enzyme is affecting the reaction ate by this relationship. The best a choice of p H is given by H plus equal to root of k 1 plus k 2. But, whatever that p H e minus by e naught is given by this relationship. Therefore, the V M value that is the appropriate you have reaction is given by not k 2 by e naught. But, k 2 e naught multiplied by y, where y is e minus by e naught.

And this y takes the best value when we choose p H as root of k 1 plus k 2. What we are now saying is that, this whole thing is analogues two substrate kind of inhibition. Because, it is substrate inhibition with uses ((Refer time: 55:24)) this kind of relationship.

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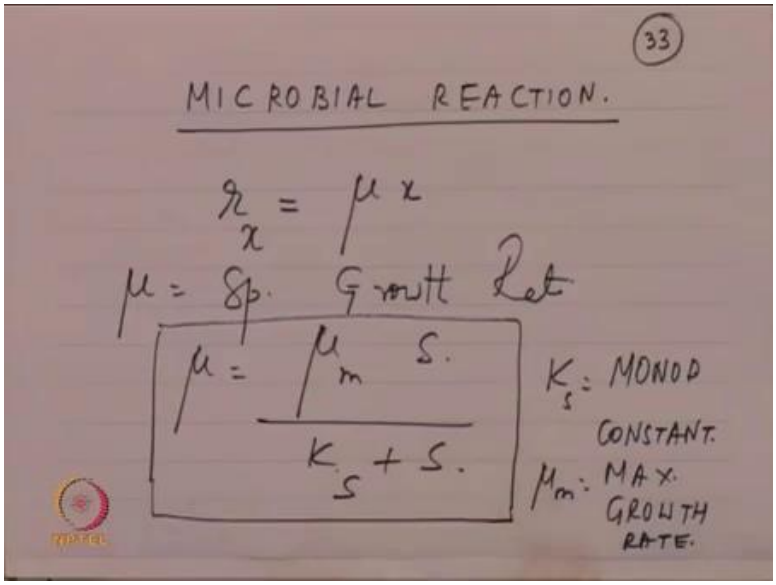

In a having set this, suppose we have a microbial reaction. We have done in this enzyme reaction. Because, we really want to see how must we can go forward and understand microbial reactions. See, microbial reactions the rate at which the micro organism will were is frequently given in this form where mu is specific growth ret. And this mu is often written in this form. Notice this is the same form ((Refer Time: 56:08)) in which Mycale is mental as written. V M is maximum velocity, K M Mycale is constant.

Here it is mu m by k s plus s. The reason for doing this is because microbial reactions are also regulated by enzymes. Therefore, we think the form will not be different. Whatever, form enzyme gives same kind of form it will expect in micro will reaction all of it. What is mu m, mu m is called the maximum velocity, k is called Monod constant. So, k s is called as Monod constant. Mu m is maximum growth ret. In enzyme literature ((Refer Time: 56:58)) V M is called as maximum velocity k M is called as Mycale is constant.

In a microbial reactions we call it as mu m maximum growth ret and k s as Monod constant, it is very analogues.

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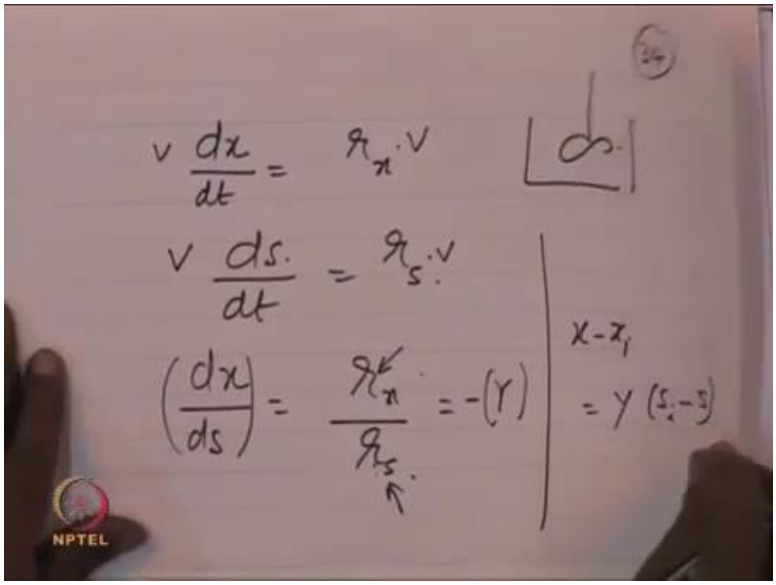

Now, if you conducting this reaction let us say, we write v times d x times d t is a r x in a batch vessel let us say, in a batch vessel. And then, we have v times some in a d x d t is r s. Therefore, we should have d x to d s r x divided by r s. What is the r x, ret of growth of cell mass, the rate of substrate consumption. Therefore, now this ratio r x to r s what meaning can be give r x to r s. In the literature they give it a meaning of yield coefficient. This ratio is called as yield co efficient.

That means, whatever is the substrate that is consume, how much of that substrate goes towards growth of cells. If we have spending you are a substrate and growing this cells this ratio is what is the yield co efficient. Now, x is growing s getting consumed therefore, we put an negative sign other word. Because, x is growth x is consumption. Now, you can integrate you can integrate this I will write here. So, it becomes x minus of

x i equal to y times s i minus of s. I will write it big in it where it cannot see properly.

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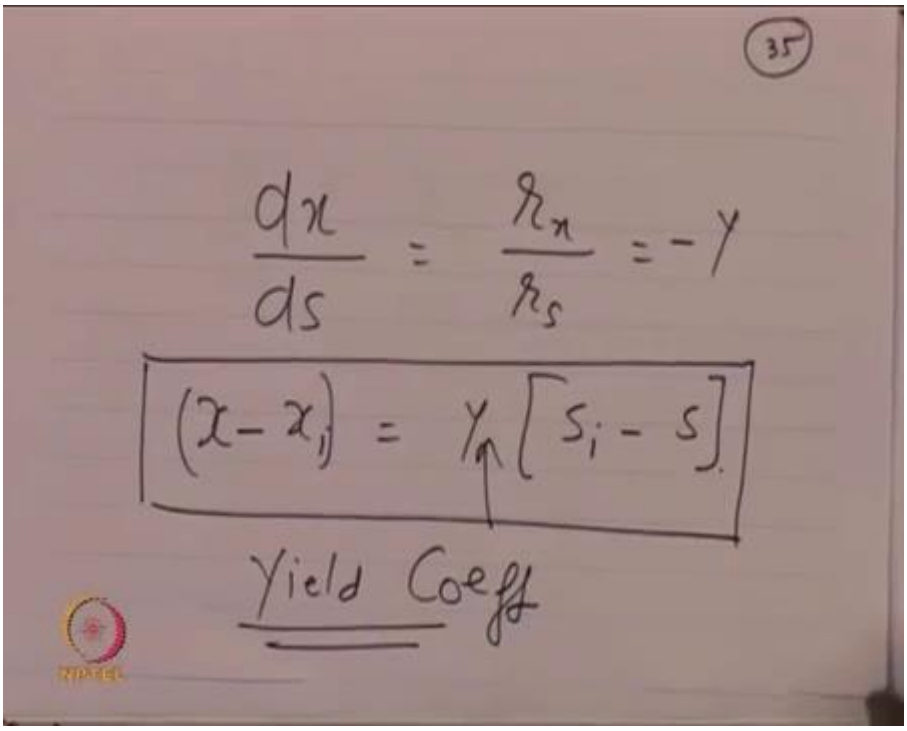

I write it again. So, d x d s equal to r x by r s integrating this, you get x minus of x i equal to y times s i minus of s. Yes or no, I put a minus y here. So, let we can this. At now, let us go back to this equation ((Refer Time: 59:20)) r x times v. So, we want integrate this equation. We want to integrate this.

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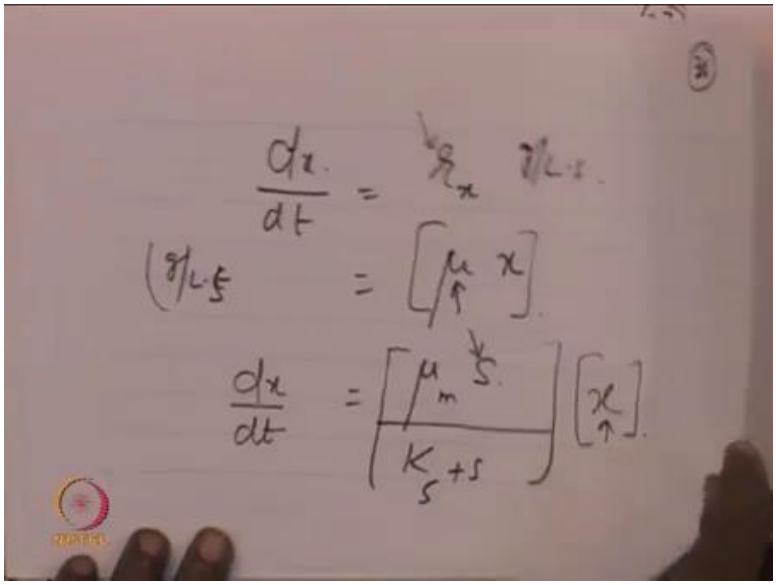

So, d x d t is r x, this r x we said is mu times x. Now, mu we said this is grams per litre per time second, this is also grams per litre per second. It is squared alright. Now, it is mu m s divided by k s plus s. Now, r x we said in enzyme literature to microbial literature, what we are saying is that the rate at which the cells are growing is given by this representation. The rate at which cells are growing ret mu specific growth ret multiplied by this cell concentration.

This specific growth ret this depends upon the environment in the cell is growing. That environment is describe by this concentration s. mu s by k s plus s is the rate at which growing to which you multiplied by this cell concentration to give you. So, this is d x d t. Yes or no. Now, how do you know integrate this. I have to replace the x in terms of s. I have to replace s in terms of x, for which we have derive this relationship ((Refer Time: 60:48)), where we said y is called the yield coefficient, this is yield coefficient.

Now, this is yield coefficient ask you self, when is it that the consumption of substrate to growth of the cell. Why should it be constant, it is constant or it may not if you and I for example, beyond in a if we do not growing consumes substrate, but we do not growth correct. On other words, this ratio this depends upon the age of the cell. Therefore, it may be constant when may not be constant.

So, the fact that we have assumed to be a constant implies that when we have apply this to a particular problem will have to see whether, to update this why as we do the integration, if you know the either cell. Otherwise, we use the mean value, so there it is the applies for the entire time duration for which do the integration. So, this number y is an experimental quantity. So, that would be available to you. Assuming that is the constant now we can. So, let us replace this y I will do it next one.

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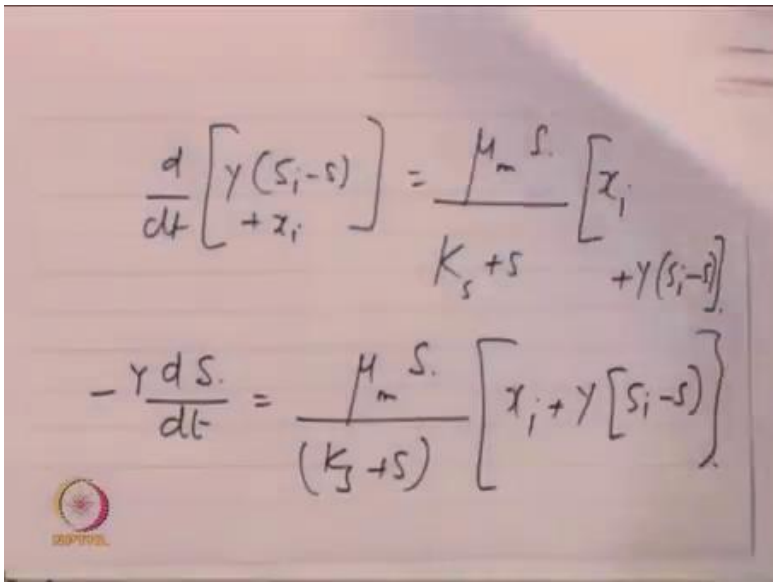

So, you have d x d by d t of what is the x y times s i minus of s equal to mu m s divided by k s plus s. What is y, this is x i plus y times s i minus of s. So, left hand side becomes d s d t with the minus sign. Right hand side becomes mu m s by k s plus s. And this is x i plus y times s i minus of s. Now of course, this can be integrated in all that. So, I write the integrated form on waste time on this.

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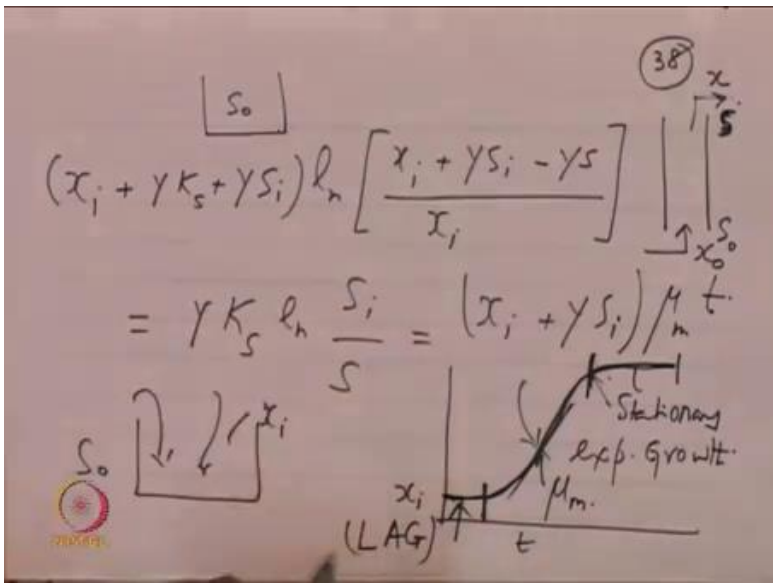

Integrated form look like this x i y k s y s i l n x i y s i y s divided by x i equal to y k s l n s i by s equal to x I y s i mu m times t. This how the integrate form looks like fairly

elementary integrations of. Now, the context is the following, if you a growing a cell, you start with certain value for x i; that means, you have a vessel in which you put your substrate. And you put your cell small amount of cell x i and cells starts growing. So, when the cell starts growing what you see is this. This how the experiment looks like this.

This cell about what is called as batch growth curve generally, gives you 1, 2, 3 and 4 distinct regions. And region during which the growth is very small. This is what is called as the lag phase. What is the lag phase? It is the phase of growth of this cell during, which you do not see much change in this cell density. So, that is the period during, which the organisms present in your environment is trying to adjust itself to the environment of the growth.

So, it takes to some time to understand the environment to adjust and to I am recognize and so on. Once it is an understood the environment start growing fairly rapidly. So, this is what is called as the exponential growth phase during which period the organisms grows fairly rapidly. You can see here in a this region, this slope is something like mu m grow is very rapidly. And after the point to find that the growth start to the slope down. Some way here the growth starts to slope down.

How do we explain this slowing down on growth, people explain this slowing down of growth in various phase. Once simple explanation that this substrate and run out. There is not enough substrate and the result you are rate function here mu I am x by k s plus s is come down, the rate is come down. So, there various other explanations in the literature s very elementary kind of explanation is the substrate of as decreasing in therefore, you see a decreasing the rate at which the growth as taking place.

And after a point, you find the there is no growth we see. And this is the explain by saying that this substrate is exhausted. There is no substrate on other words s is 0. Therefore, there is no growth. So, the a batch growth that you will see typically, has 1, 2 and 3 phases, lag phase, exponential phase and the stationary phase. Let us, look at this once again.

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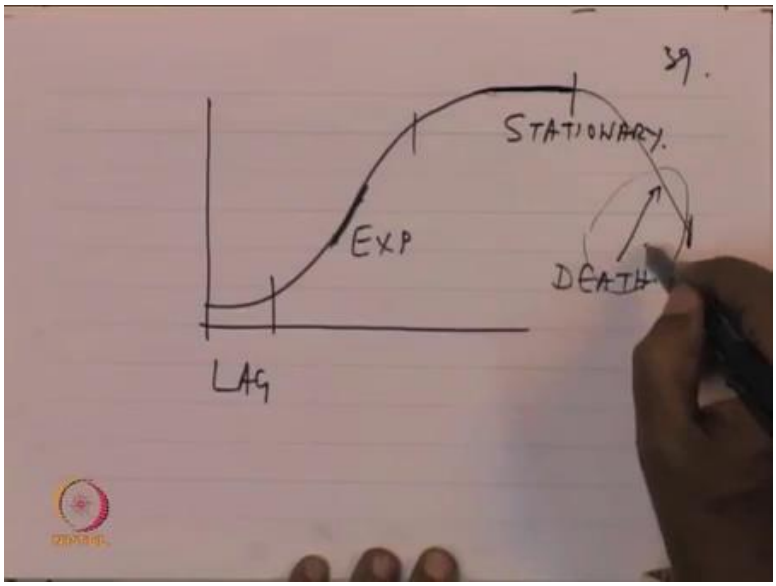

This is lag exponential. And then this is stationary. Now, if you go to an industry you will find that another phase, which seems to be have interesting to them, which is a phase what is called as death phase. Now, tell me when is it that a you know, the user is very you know to understand this phase of are the organism, which process of a process industry this phase of the organism of very great importance. You growing the organism exponential growth stationary phase.

And then is the death phase. Now, if you go to penicillin factor for it for example, what are there in interest. There interest is to make as meant penicillin s as possible. Now, what do we start the start with the in areola. And make that organism to grow. After it grows and reaches sufficiently high density. What they would do is that, they would not provide a proper environment for it grow further, but produce penicillin.

Let me, the conditions required to produce penicillin at different from condition required for growing the organism. So, appropriately they will provide the feet are the substrate, at a rate that it is sufficient for it to make penicillin only it will not grow further. This is that means, this phase of growth is useful for producing secondary metabolites. This phase is useful when we are trying to make the organism for sale. Say, bake or cease if you want to make. You want to be in this region.

Because, you want to growth organism, because you want to the cell the organism. If want to make penicillin you want to organism as to be in this region. So, that little

produce penicillin. Suppose, you are interest is actually to disinfect the process. See, suppose the entire process disinfection, disinfection is the huge activity in fermentation industry, it is in the water treatment in the industry.

Then here you are looking at this phase, because you are trying to understand what is the condition required to kill the organism. So, the death phase is what a would be have great consent to you. On other words, what we are saying is that two biologist. The lag phase, the exponential phase, the stationary phase the tells we are important. Because, different process required different aspects of the growth to be of importance to him.

Now, suppose instead of conducting this reaction in a batch equipment with s naught I conduct this reaction in a flow equipment. In the sense you make it flow in upwards on downwards as for as this form is concerned makes no difference. As long as, you know what is see this is the exercise initial cell concentration. Here, it will be initial cell may be x naught. Continuously, it is cells are coming in, continuously cells are going out.

Or in a flow process it is a in what is called the tower fermentation for example, instead of batch fermentation. Continuous as cells are coming in cell of growing and you get products out cells out. So, you are substrate get consumed from s naught it get becomes s to the extend it just consume the product is produces. So, a slow process and batch process we know a equivalent, as only thing wherever t appears if pride residence time. Otherwise it is this is no difference, is at clear.

So, as for as the growth process is concerned is governed by, you are Monod constant k s, you are the yield co efficient y. And you are maximum growth ret mu m k s and y are your growth parameters. But, there is one fundamental between batch and continuous in biology. In batch process what happens is that, the organisms get a lot of time to adjust to the environment. Why in flow process they do not get that kind of time. So, you will find biology flow process do not work as well as in batch process.

So, this is the very fundamental difference. And therefore, the parameter that we have determining from batch process equipment may not generally applied to flow processes. What is mu m k s and y in a batch process. And the mu m k s and y in a continue may not the this same. Because, the fact that the organism do not like this kind of environment. They like to work in the an environment, which there I have adjusted. Now, there is an interesting feature that you will is not common.

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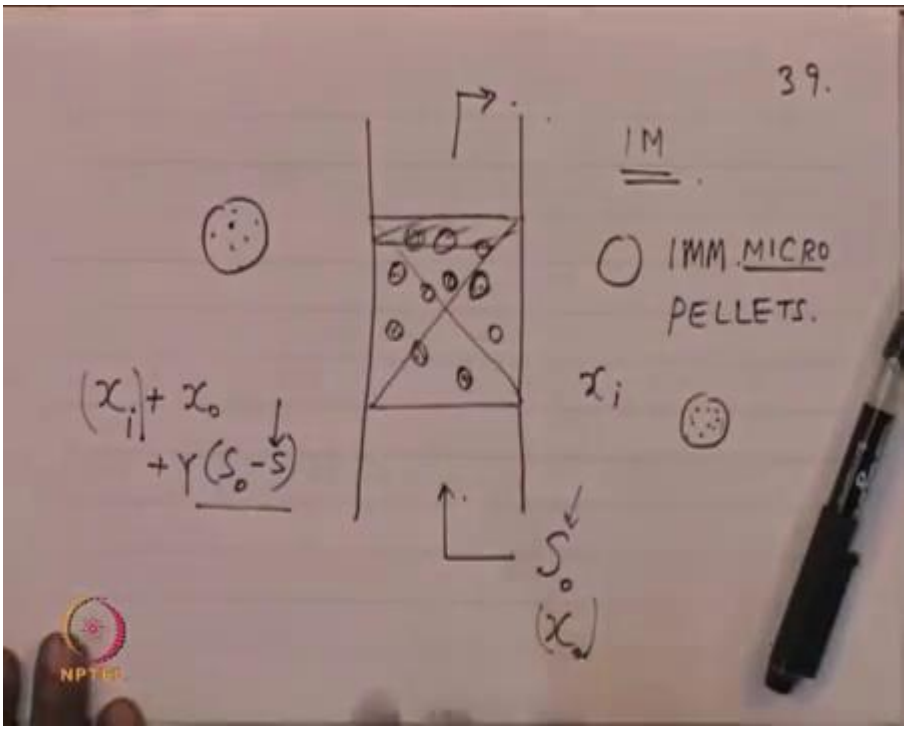

But, lot of literature that is available, see what see there is the great interest at particularly in fermentation industry to increase the through put. And I mean in these there for all of us you know, for given volume can be reproduced more. Now, what people have done is that they have created what is called as immobilized enzyme pellets, immobilized pellets. What are immobilized enzyme pellets, you take the is not immobilize microbial pellets.

Now, this organism what they do we take it gel. And then immobilized the organism inside the gel, where it types of gel is available in which you can immobilize the organism. So, what is it that you have now instead of having micro organisms, which is suspended which is now immobilized inside the matrix like a heterogeneous catalyst. Something that we have seen is an industry is a heterogeneous catalyst. Now, you can put this immobilized catalyst in here.

So, what is that contain, it contains your micro organism. Now, you have your substrate coming in, it may coming which some cells it may not come in made some cells. That is, but all these have got x i grams of cell inside. Now, as this substrate goes through what would happen, as substrate context this spell x this is get consumed. Yes or no. So, what is the concentration of cell at any point, it will be what we have put in, plus what comes

in, plus what grows.

Do we agree? x i is the cells that I have put inside this per unit volume. See what is called immobilized cell reactor, what we do is the take the organism and put it inside a pellet. Now, what you have is something like a heterogeneous catalyst. You can pack up you are reactor with these catalyst. Now, you are substrate is coming in s naught it may also bring some cells, it may, it may not on the other. But, if we look at any cross section of the reactor you have x i grams per liter of immobilized cells.

You had x naught coming in plus, so much is growing. Because, if fact that the s naught become has become s. On other words, that every cross section of the equipment the concentration of cells is x i plus x naught plus what has grown, because of the reaction.

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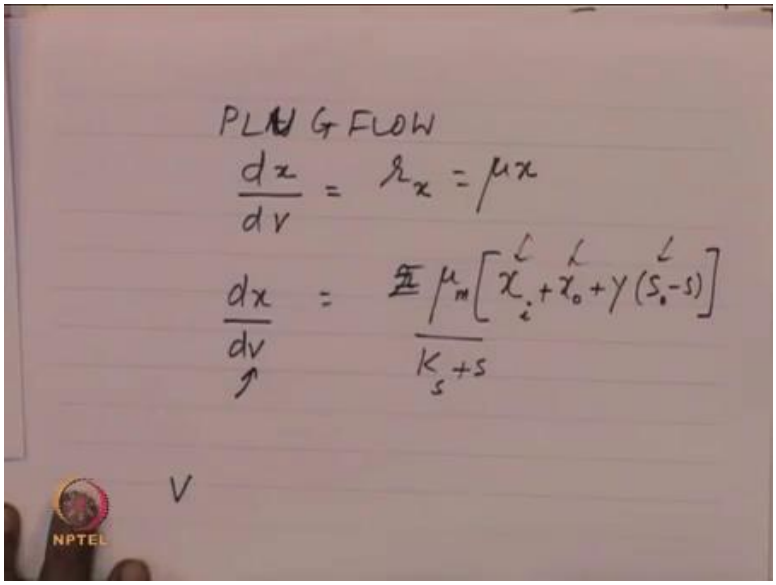

Therefore, in our equation here d x d v equal to r x. What is this r x, mu times x, this x previously now it is become, do see this point I am come m come. Do you agree with this? The right hand side, this is mu x previously, now that x is has all these extra features coming in. Yes or no?

Student: ((Refer Time: 74:47))

I am talk about the continuous process say, it is the plug flow vessels I say, it is the continues process. Now, what is this mu? Yes or no, What is this v, it is the volume, ((Refer Time: 75:15)) when we did not have this immobilize cells here. The liquid as to

going write through, therefore the volume that is the available for reaction if it is v previously, now it is the occupied certain amount of spaces loss, because of this.

Therefore, the actual liquid volume now previously, it was fully now it is v times 1 minus epsilon g times epsilon l, where epsilon g is the gas what is called gas hold up. Because, the reaction rate of very high the lot of gas, which is held up in the equipment. Epsilon l is the liquid holed up previously, Epsilon g 1 0 epsilon l as 0. Because, the reaction rates for like that. Now, because ((Refer Time: 75:54)) of the fact that we have put in this immobilize cells, we have loss some of the reactor volume.

Because, of Epsilon g n epsilon l. So, the left hand side has to multiplied by 1 minus of Epsilon g n, Epsilon l. You understand is clear to all of us ok. So, now let us look, back at what we have done. This is what we did and for which we wrote this solution somewhere, I will write it I will have it some way here solution is there. Please recall this solution for a batch equipment ((Refer Time: 76:24)) for which what we do is say. We said t is the time for reaction.

What is previously we had put in x I, x i these cells in the batch equipment. Now, it is the flow equipment, flow equipment the feed is coming in with the x naught. We have putting so much of x i, x naught is coming in. So, much is growing. So, the effect of all this is to increase the rate at which chemical reactions takes place. So, you will find that immobilize cells I mean a huge amount of literature exist between in 1970’s and 1980’s by immobilize cells was investigated as the possible, where increasing reaction rates in biological react.

Huge amount of integer exist. And the only thing that we have to recognize is that whatever, is our equations here you would appropriately change these this take in to account these effects that I pointed out. But, what did turn out is the following. That many of these great ideas some have in the industry it did not work out very well. Let me, write the final form and then me explain why?

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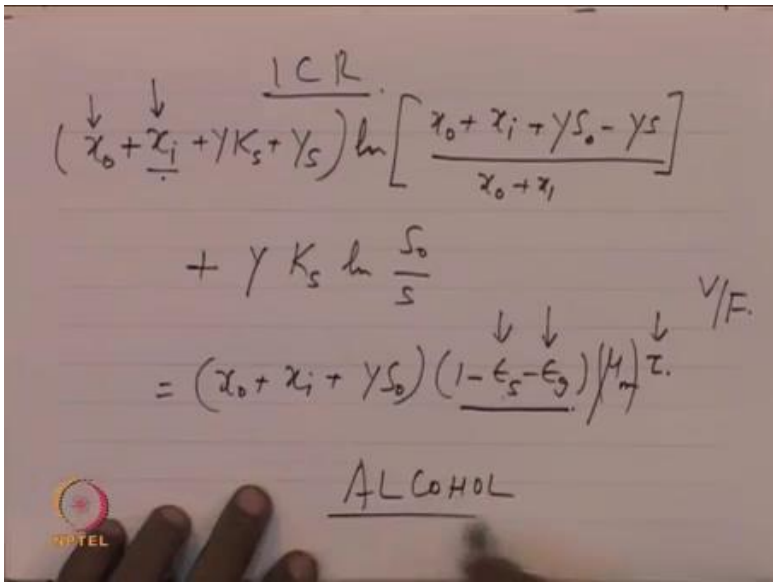

So, if we have immobilize cells your answers look like this. See, the form of this is identical to what we have already written. So, I am not writing it again. So, this is for a immobilize cell reactor. There is understand quickly. x naught is the continuous input of cells. x i is the cells that you have put in, because you have immobilize cells as put in inside the equipment. And now, Epsilon s in Epsilon g is holed up of solids holed up of gas in the immobilize cell equipment.

And tau is the residence time, which is V by F. This is at all microbial parameters. Now, the rate at which the reaction that means, the extent to which we can drives at reaction really depends upon, how much you will lose, because of this. How much of gain because of this. This is the loss, because you are losing volume. But, this is a gain correct, because you putting so much of cells, and you loss so much of volume. So, we plus point of this and minus point of this will have to see how it affects your process.

They experience that is reported them in the literature is that it is substantial, it is not small. And that is how this huge amount of certain got done particularly, for alcohol. Huge amount of alcohol fermentation, huge amount of research exist. Now, having set this what seem to happen is that, this particular ((Refer Time: 79:37)) immobilize cell, in which the cell immobilize. As the reaction takes place, for is the alcoholic fermentation for all you know lot of carbon dioxide is produced correct.

Now, this carbon dioxide, which is produce inside this immobilize cell it may to come

out correct. Now, as carbon dioxide is come out, because the huge increases volume, it is ruches these immobilize cell. So, the what people formed was that that these stability of the immobilization was the unsatisfactory. And consequently the net result that the anticipated. Because, of the immobilization did not eventuate in a process. It work very well in the laboratory in all that. But, in a process it did not work all very well. Therefore, immobilize cell react is not very popular, or not very common in the process industry. It will work very well in the laboratory, will stop there.