Course on Industrial Biotechnology
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Lecture 12
Enzymatic Reaction Kinetics (Contd.)

Now in the last lecture class I discuss about the determination of the Kinetic parameters of enzymatic reaction and now I am going to discuss one important parameters associated with the enzymatic reaction.

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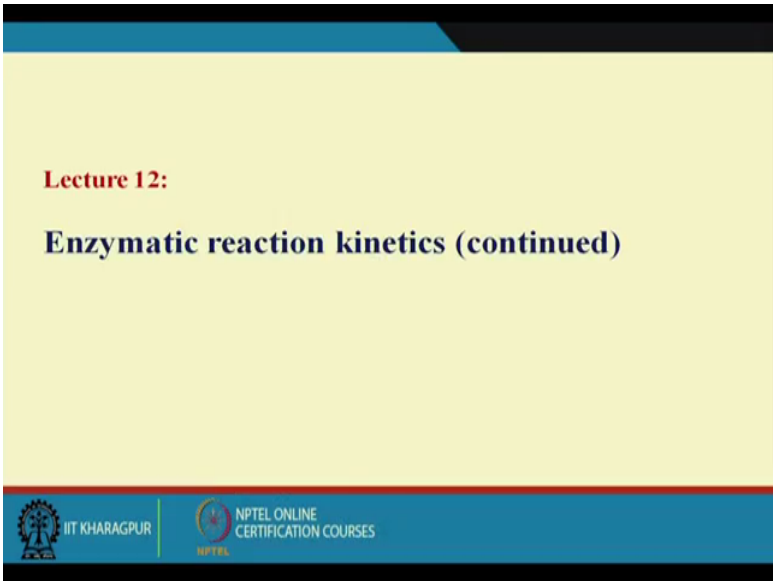

That is the inhibition now question comes what do you mean by inhibition?

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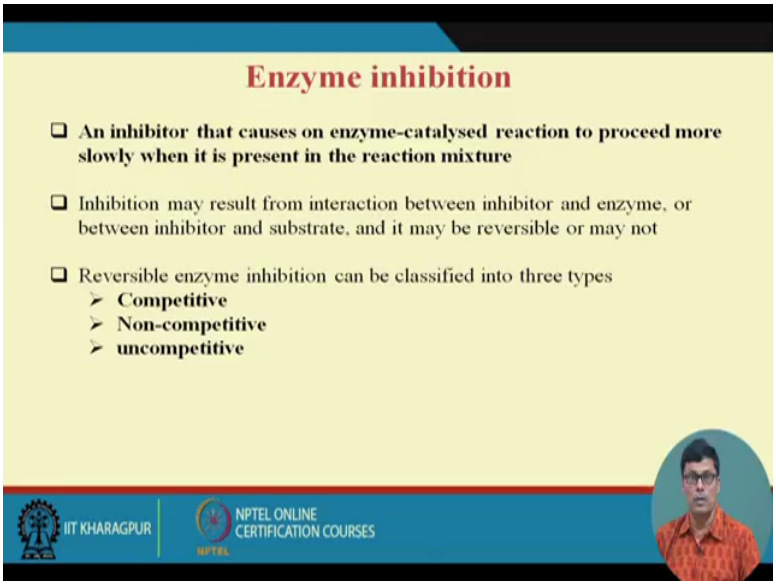

Inhibition means retardation of velocity of reaction is nothing but the tradition of the city of reaction and inhibitor causes the enzyme catalytic reaction to proceed more slowly when it is present in the reaction mixture that means that innovation doesn't means of stopping the reaction is reduced the rate of reaction that is that is inhibition and inhibition may results from the interaction between the inhibitor.

And enzyme or between inverter and substrate and it may be reversible or may not be reversible. The reversible enzymatic inhibitor can be classified into 3 types 1 is called competitive inhibition another is non-competitive inhibition and there is uncompetitive inhibition.

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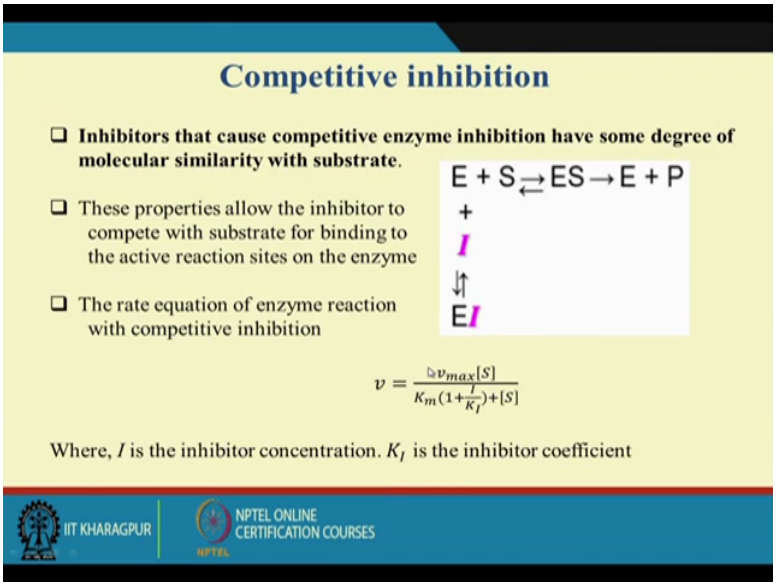

Now let me explain what do you mean by competitive elevations? Competitive elevations means inhibitors that causes competitive enzyme inhibition has some degree of molecular similarity with the substance. No what do you mean by that?

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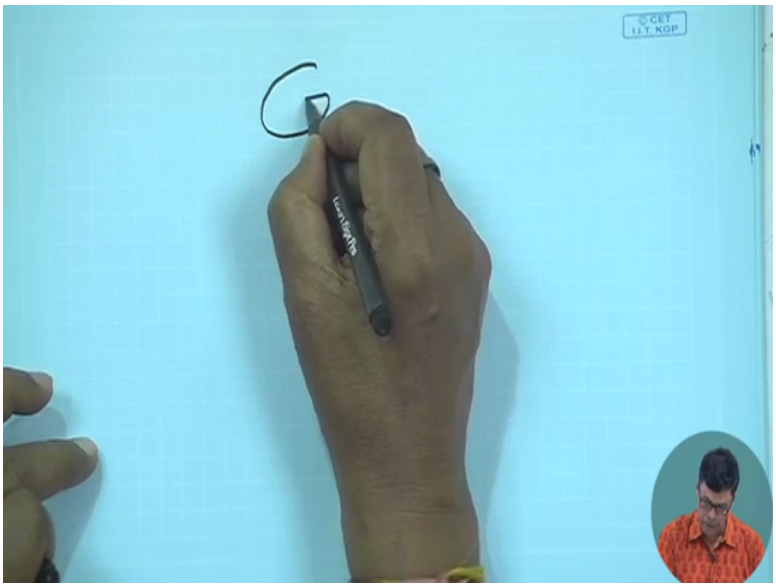

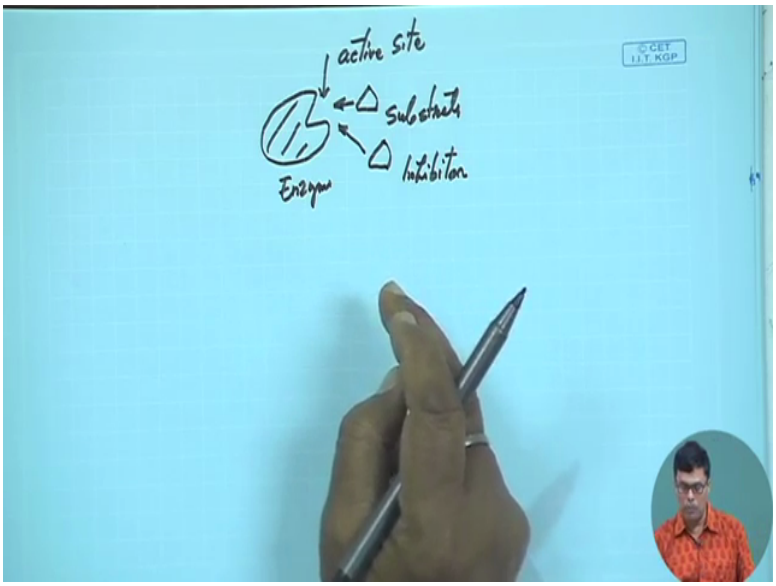

Suppose this is your enzyme and this is active site this week all considered as a active site and this is the inactive site ok. Now if you are substrate configuration this is substrate this is the enzyme. Is like this so substrate will see that the active site and give the product. Now inhibitor if it has the same configuration as the as as compared to the substrate the configuration of the inhibitor.

And substrate they are (())(2:39) so that they are not there they have resemblance with each other so that they both came compete with the same active site you see there competing for the same active site. This this is called competitive inhibition.

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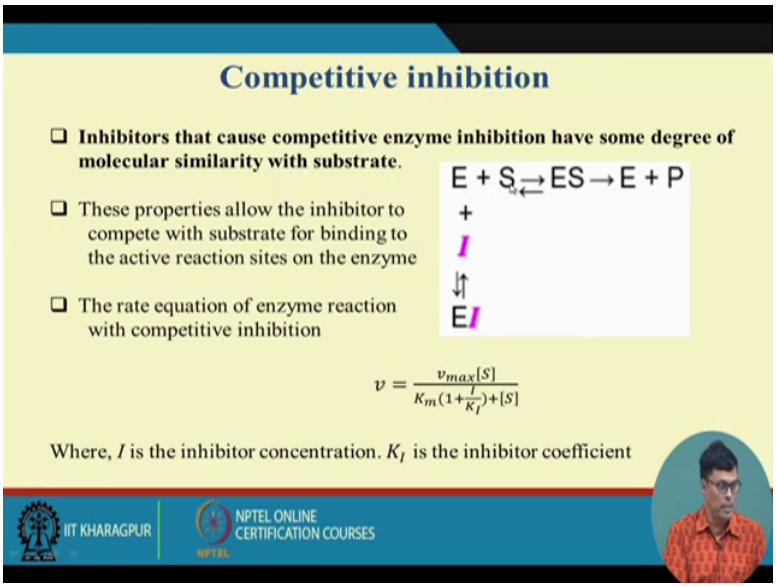

So here in this enzymatic reaction you see E plus S give ES and then it produce E plus P and at the same time plus I produce EI when EI formation is there is no product formation but when there is a ES formation enzyme substrate complex formation is there there is a product formation so this is how inhibition takes place that it is part of the enzyme goes inactivated with the help of inhibitor that is why rate a rate of reaction decreases.

This property is allow the inhibitor to compete with the substrate for binding to active reaction site on the enzymes and rate equation of the enzyme reaction with competitive inhibition can be expressed like this now if you if you analyse this reaction this is called KI. KI is the equilibrium constant of this steps.

Now if we if we analyse this this kinetics of this equation we will get derive this equation now here 1 interesting thing this is equal to V max S KM 1 plus I plus KI plus S. Now if I put I equal to 0 then it will be becoming the Michaels menten equation you can see that if we put I equal to 0 then it will be becoming the Michaels menten equation.

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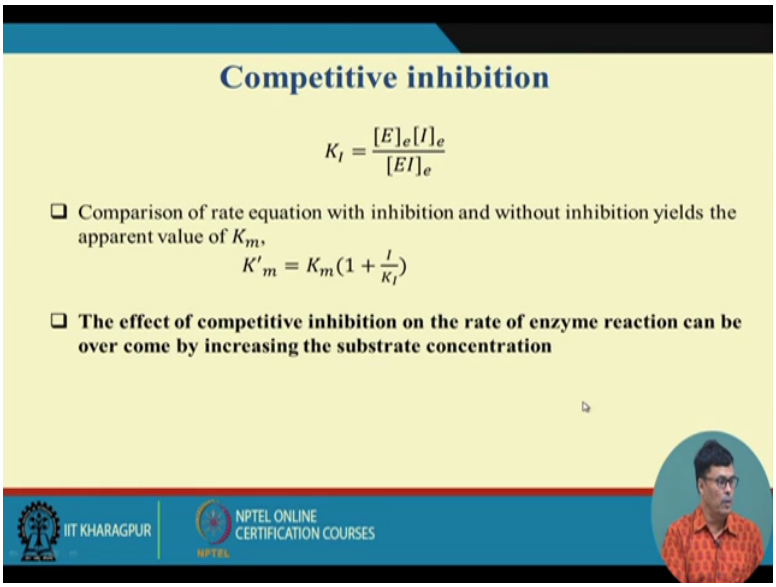

So now K K this this show what we have that K incase of Michaels menten equation this is this is KM now this has been modified as KM into 1 plus I by KI that is exactly what we have written here this is modified by this. The effect of competitive inhibition on the rate of reaction can be over come by increasing substrate concentration. I can I can give you very simple example here.

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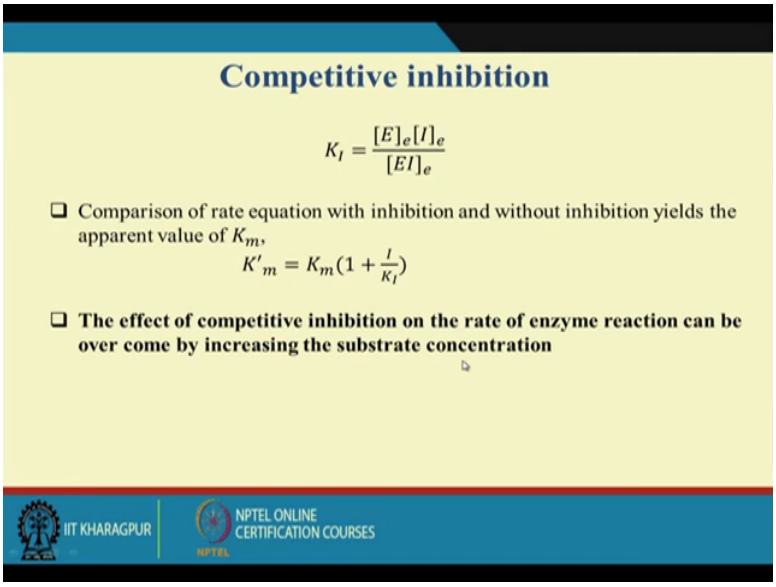

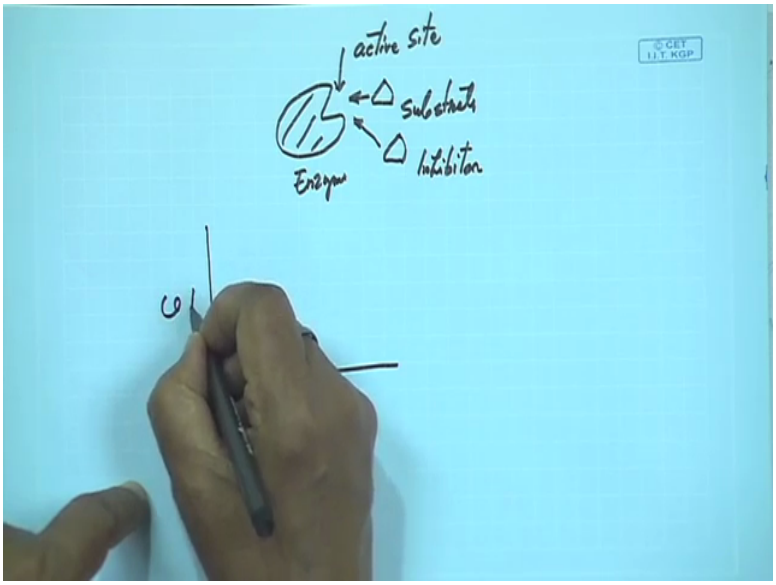

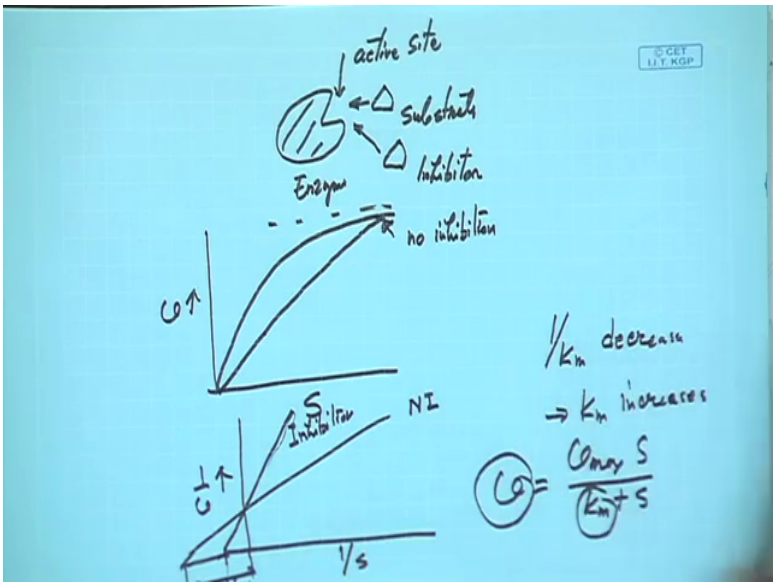

Suppose we have V is velocity of reaction and S is the substrate and this is the co relation we have this is no inhibition. Now in case of competitive inhibition what will happen that you know your rate of reaction will increase but has the infinite time it will go to the V max. So this is your V max now so if you if you plot live (())(5:32) plot 1 by V buses 1 by S if you plot this is no inhibition no inhibition ok.

But in case of inhibition what is the plot will be like this that is this intercept I should you the. What is this intercept this is equal to minus one by KM so 1 by KM value decreases as this is inhibition this is inhibition so in case of inhibition competitive inhibition that this is 1 by KM decreases if 1 by km decreases means what?

When came increases am I right? Came increases the if KM increases now if you have the we have the Michaels menten equation Vmax is KM plus S now if KM increases velocity of the reaction will decrease. This is how the velocity of reaction decreases in case of competitive inhibition.

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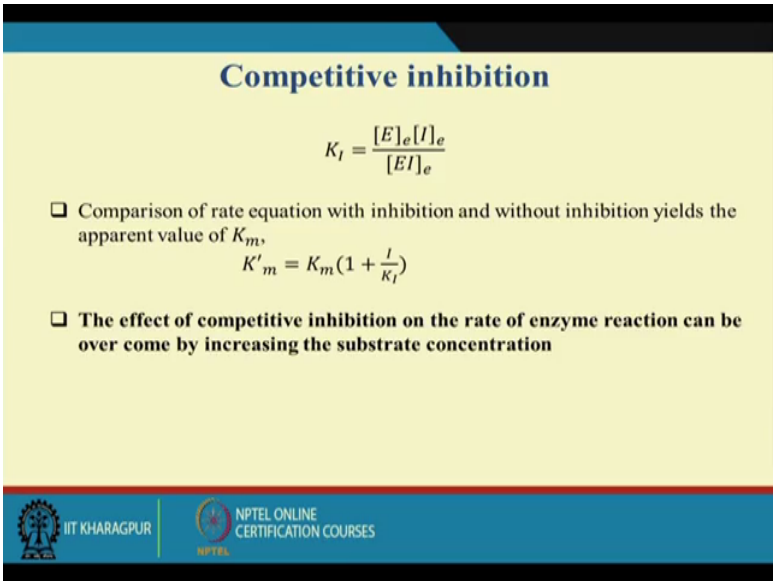

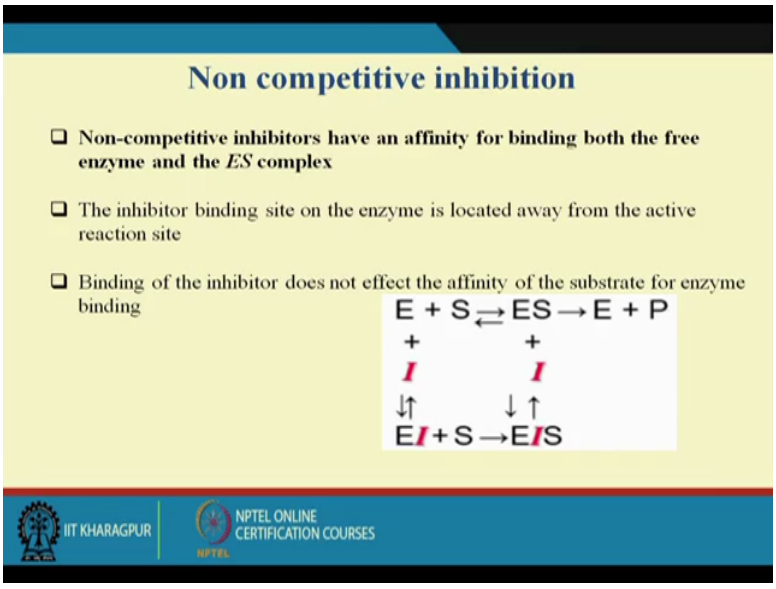

Now next le let me discuss about the non competitive inhibition in case of non competitive inhibition the inhibitor has an affinity for binding both free enzyme and the ES complex now it is very interesting E plus S produce ES also ES can bind with I that is the inhibitor form EIS complex.

And E plus I form EI complex and EI can form a bind with S EIS complex and this is called trimolecular complex. So I can I can I can I can so in case of non competitive inhibition how it is the how is it take place?

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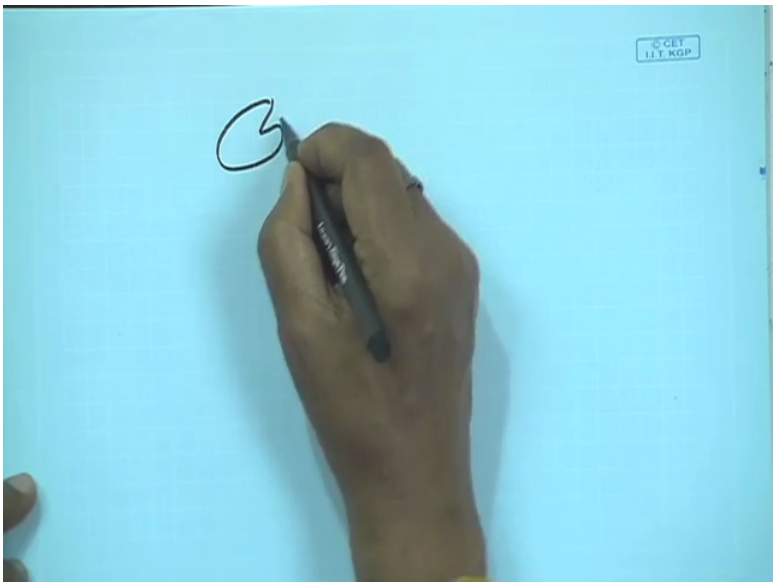

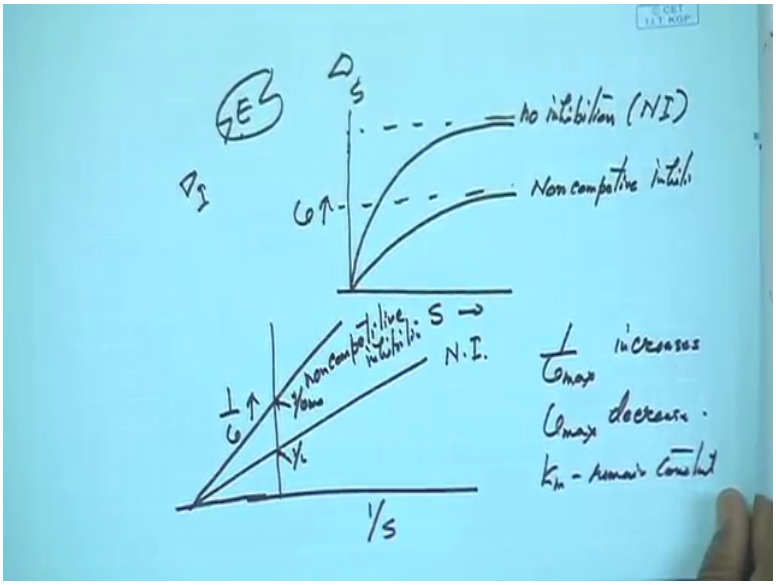

Suppose this is this is your enzyme and this is your substrate and this is another active sites so this is inhibitor and this is your substrate the substrate bind here inhibitor (())(7:53) like this you know that that they form the tri molecular complex so you have IES that is tri molecular complex formation is there. So in case of non competitive inhibition there is a possibility of formation of tri molecule Complex now here if you plot V by S.

This will be no inhibition. But in case of com non competitive inhibition the plot will be like that means you see the Vmax value changes is here the Vmax value and this Vmax value is changes. Now if you if you have the line of (())(8:53) plot 1by V versus 1 by S if you plot this will be no inhibition this is no inhibition. Then in case of competi non competitive inhibition it will be like this. This is non competitive inhibition.

So here what is this intercept we know this is 1 by Vmax am I right? So this is 1 by Vmax this is also 1 by Vmax. In case of no inhibition 1 by Vmax will be less as compared to when can there ther the the the non competitive inhibition. So in case of non competitive inhibition 1 by Vmax is increases so we can write Vmax decreases. But Km remain constant Km remain constant.

So we have seen in case of competitive inhibition Vmax is constant but in case of in non competitive inhibition Km remain constant this is the this is the thing that we have but Vmax decreases here. And as the Vmax decreases the rate of volt rate of reaction also decrease. That is how exactly the inhibition takes place.

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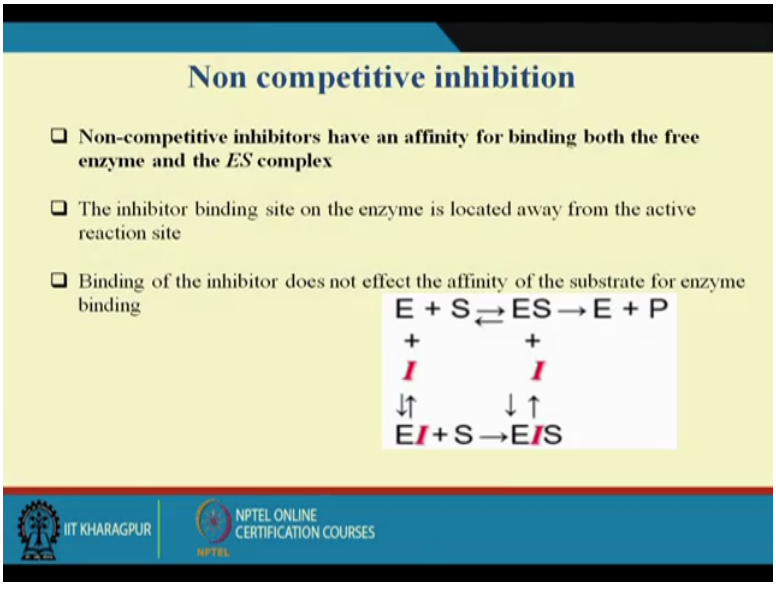

Now inhibitor binds the sited on the enzymeis located away from the active reaction site and binding inhibitor does not affect effect the affinity of substrate for enzyme binding now here I want to point to point out that a very interesting thing there is another term is that the partially non competitive inhibition and partially that competitive inhibition partially.

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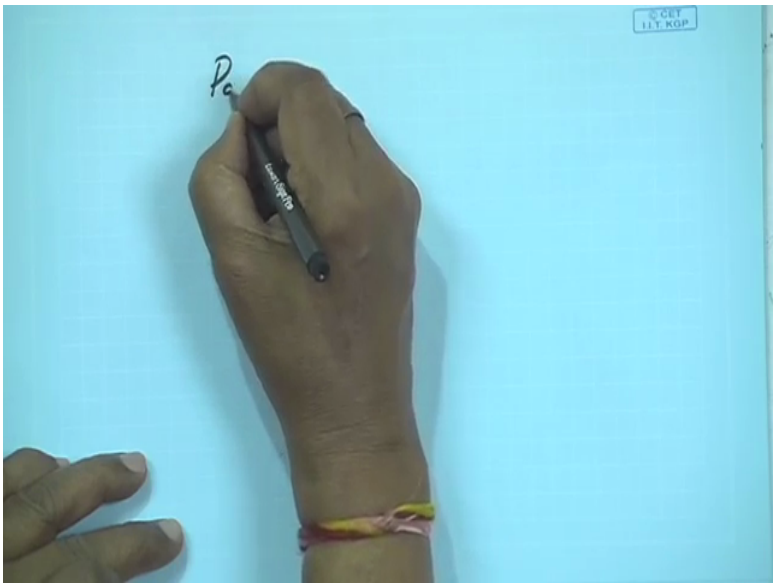

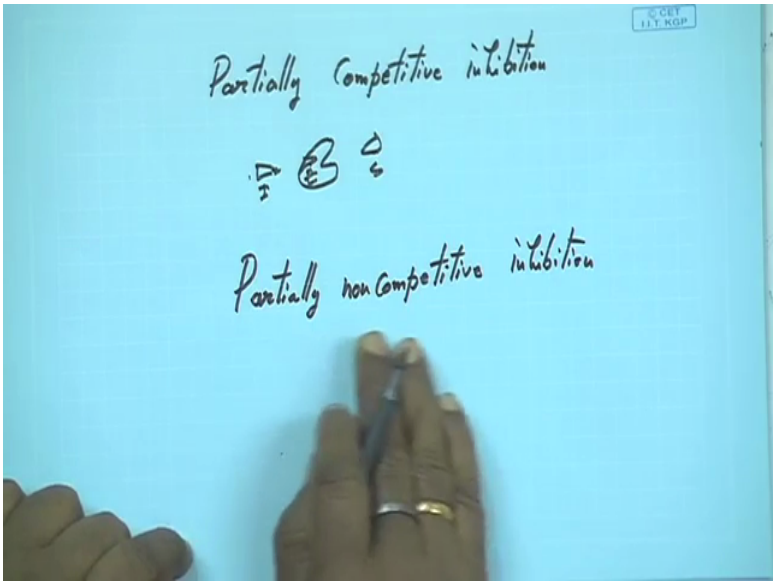

Partially competitive inhibition and partially non competitive inhibition. Let us say partially. The in case of partially competitive inhibition what is happening that I told you that we have we have this enzyme this is substrate the wa now in case of partially non competitive inhibition the inhibitor binds other than the active site because this is not the binding the active site it is inhibited binding hair like non competitive inhibition.

But when he finds it binds it make some conformational change in the active site and that you're not allowed the substrate to sit in the active site so that is how the rate of reaction increases that is a further decreases that is the that isvq that is called partially competitive

inhibition that is this is this is not directly competing with the invited not directly competing with active site it is competing other than active site.

And it binds and then it change the configuration of the active site so that substrate can receive that the active site (())(12:58) rate of reaction decreases. In case of partially non competitive inhibition if you look at here that enzyme the the.

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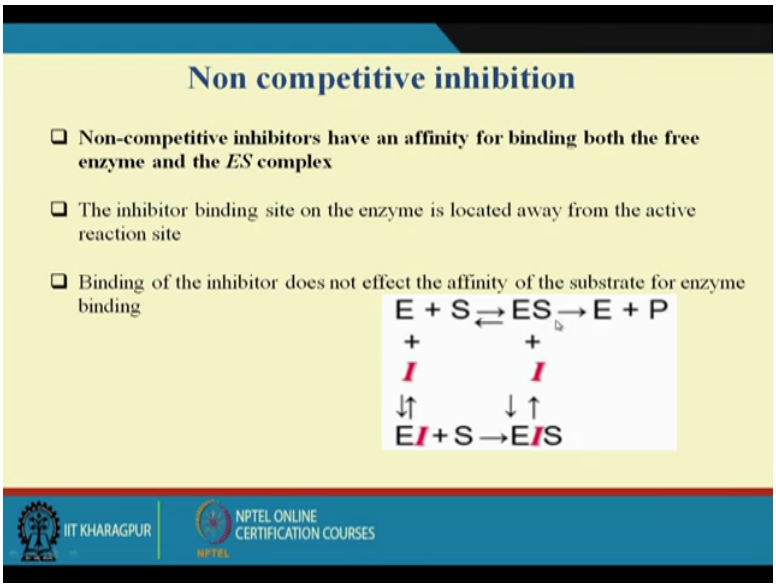

Product formation take place from ES Complex am I right? that ES equal to E plus P so but in case of partial non competitive inhibition product formation also take place from tri molecular Complex that is EIS. That also with this is called partially non competitive inhibition.

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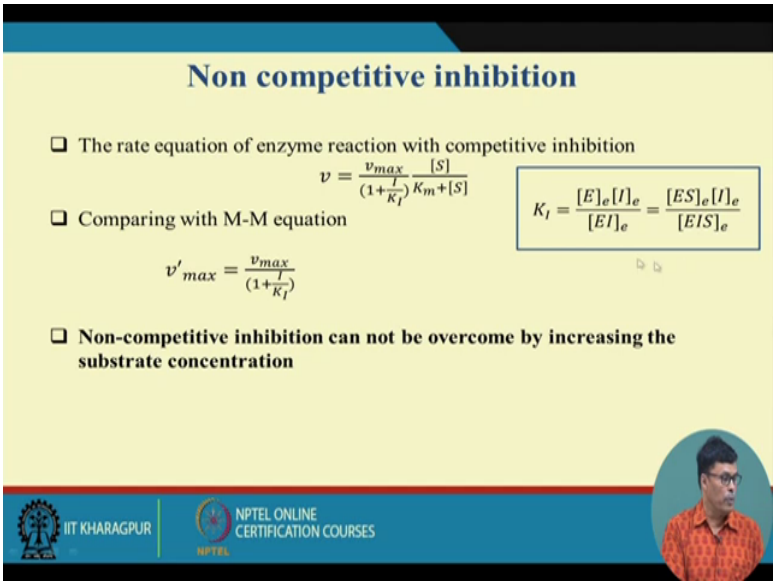

Now equation is like this that can be written like this year also if you if you (())(13:39) I equal to zero then this equation will be becoming the Michaels menten equation this is like this I equal to this.

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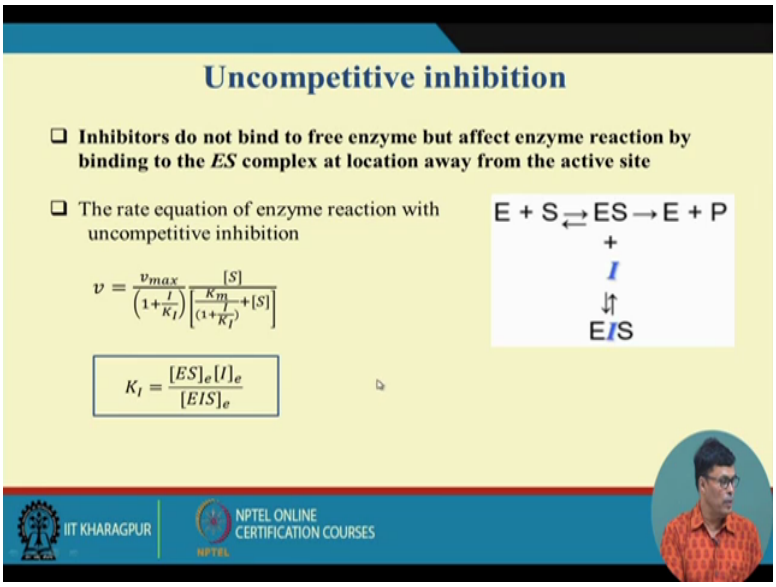

Now non competitive inhibition here this is E plus S equal to ES (())(14:04) ES plus P another ES binds with I can the tri molecular Complex and this is if this type of reaction take place then we call it un competitive inhibition and the equation is like this this can be expressed like this

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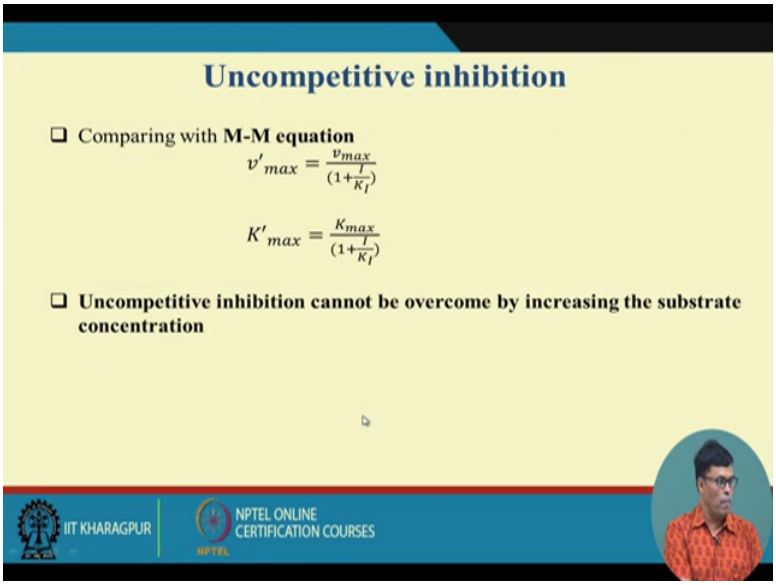

And comparing with Michaelis menten equation came value and Vmax does value will be this and uncompetitive inhibition cannot be cannot be over come by increasing substrate concentration this cannot be overcome by substrate concentration.

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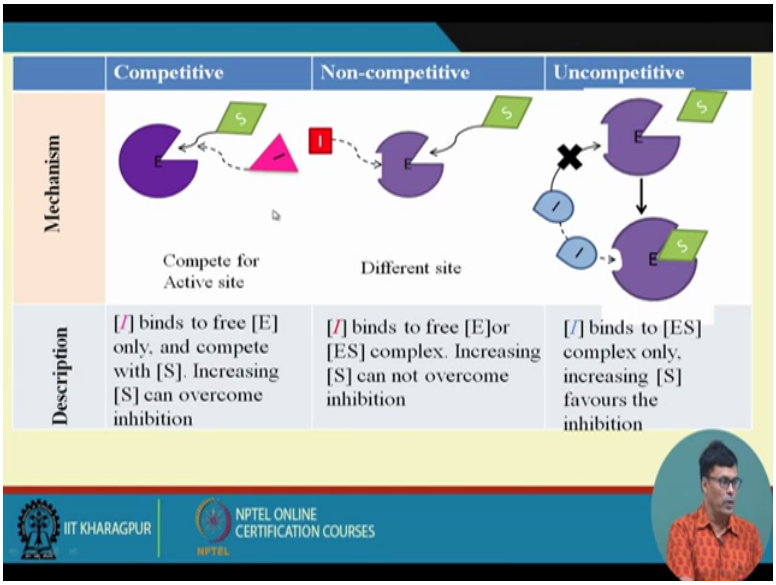

Now here the pictorially that I have already shown you that you know competitive inhibition as substrate and the innovative the complete for the same active site and a non-competitive ihibition the inhibitor binds other than the active sides.

And and then your substrate they bomb that I molecular complex uncompetitive inhibition some some cases inhibited binds some cases inhibited binds other than the active site. This is all how this uncompetitive inhibition takes place.

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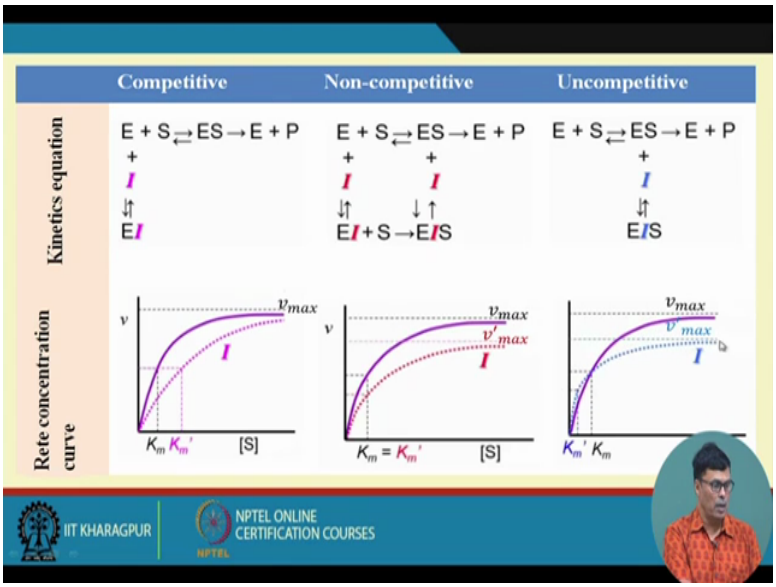

And this is this is how I have shown the reaction here the Vmax it will remain constant came increases and here Vmax remain constant came increases and and and here both the Vmax and KM changes in case of uncompetitive inhibition.

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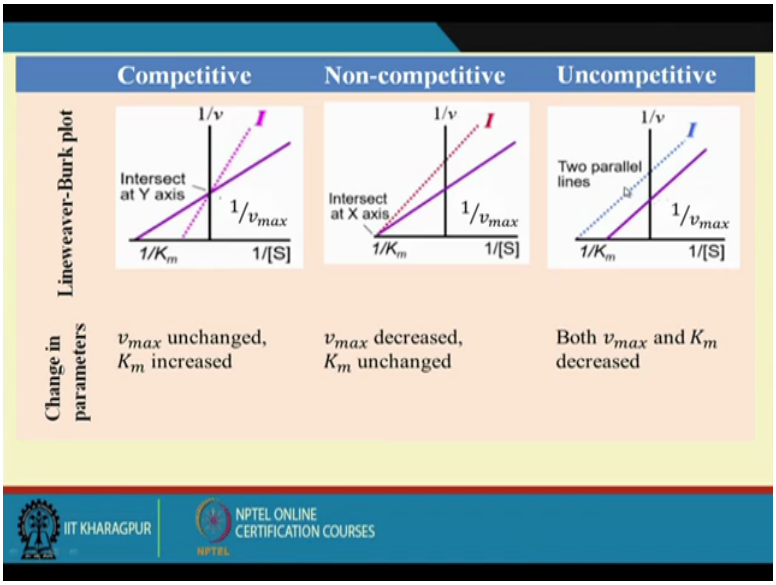

And this is the plot that we have been case of uncompetitive inhibition this is with anhibitor this is without inhibitor and this is how KM and Vmax value changes.

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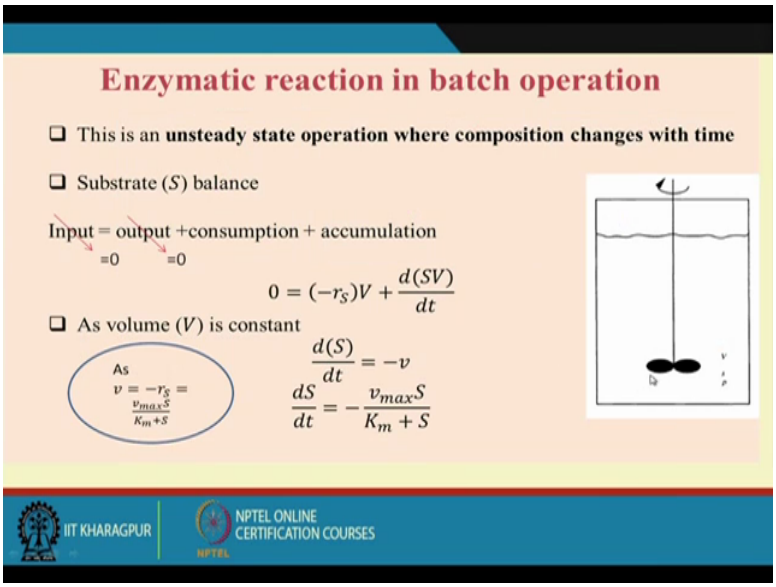

Now let us talk about we are interested to carry out some reaction in some reactor and if you want to carry out some reaction in the reactor how we can do the analysis of the reaction so so this is unsteady state operation when the composition changes with time and this is the examples of the batch operation we know in the batch system the concentration decreases with respect to time.

And has the concentration decreases your rate of the reaction also decreases so here we can write this balanced equation rate of we are doing the substrate balance rate of input of substrate equal to rate of output of substrate rate of consumption rate of accumulation rate of because since it is a batch process rate of input and rate of output should this should be equal to zero rate of consumption of substrate equal to minus RS into V.

And S SV into DT this is the accumulation of the service is the substrate concentration means the walking volume of the reactor now if you write this this is a V small V is the velocity of reaction this is one by S buy A DS by DT going to write their we know the Michaels menten equation Vmax is KM plus S that we have.

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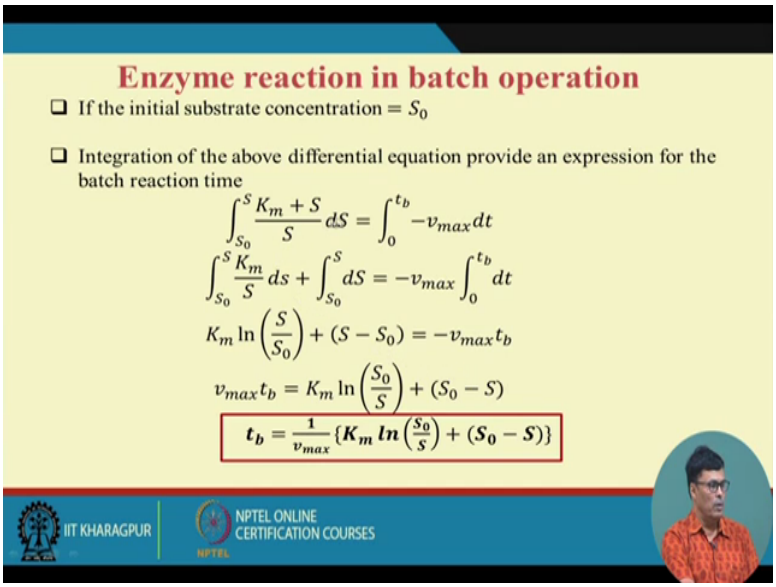

Now now we can we have we can write that this is we can take this side this this site and we can rearrange this equation like this we can we can we can the slowly slowly we can derive this because here DS by S DS by S means DLNS DLNS if you integrate S zero to S then it will be LN S by S zero and DS means S S S minus S zero this is equal to minus Vmax S is 0 to Tb Tb is the time required for the batch reaction.

So we can here have the have the equation like this Vmax into Tb equal to KM LN is zero by S Plus S zero minus S so Tb equal to we can write like this this is so here if you look at that Vmax is constant KM is constant is S zero is constant so only you have variable S so we can if you change the if you if you if you want to find out that suppose for 20% of the substrate consumption.

How much time is required I can easily calculate 30% had to have because suppose you want to confirm 90% of substrate for a particular range enzymatic reaction how much time is required? We can easily calculate with the help of the equation is very important equation you can do this equation for calculating or in an another way.

If we want to find out that after 30 minutes how much substrate is conversion substrate is converted that also we can find out after 15 minutes how much substrate is converted that also we can find out when either way we can we can never use this equation to find out the different value of T and different value of S.

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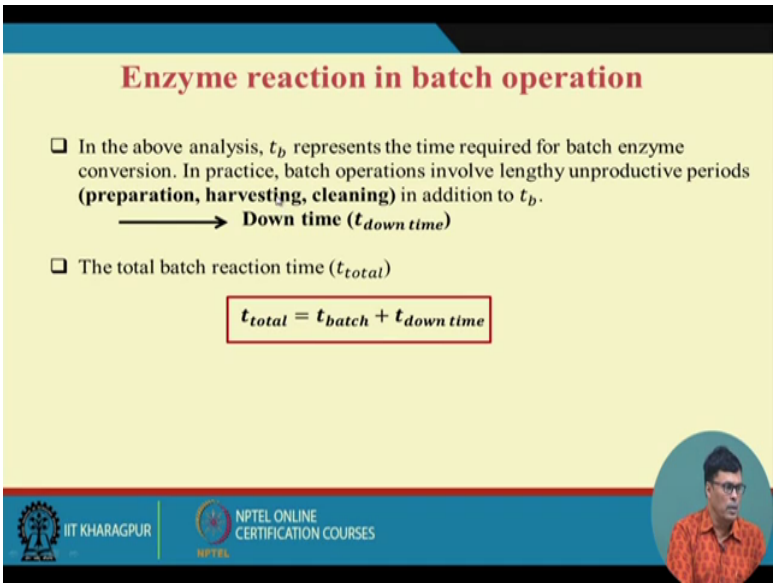

Now in a batch process I told you that in the previous lecture I mentioned that they would require some time down time and this down time is required for for different purpose for the preparation because you have to fill the vessel with the substrate you request some time then you have to harvesting harvesting means you have to take the material out from the reactor then you have to cleaning the vessel.

So all this require some time and this time we considered as they that is no reaction take place because they know that no reaction take place that is a this time is required no reaction take place so this considered that is a that is some time we call you the ideal time ideal time means no reaction take place or is considered as down time so total batch time reaction time actually T batch plus the down time.

When we consider do any kind of reactor design if you want to find out when the produce I showed you before that if you want to calculate the how much substrate is how much what should be the volume of reactor then we shall have to use this T total equal to T batch because whenever you want to calculate the volume of a batch process you have to find out.

How much product is to be produce part batch not per T per unit time particularly when you want to calculate 1 to design a battery reactor so this this total total is very important for designing the batch reactor.

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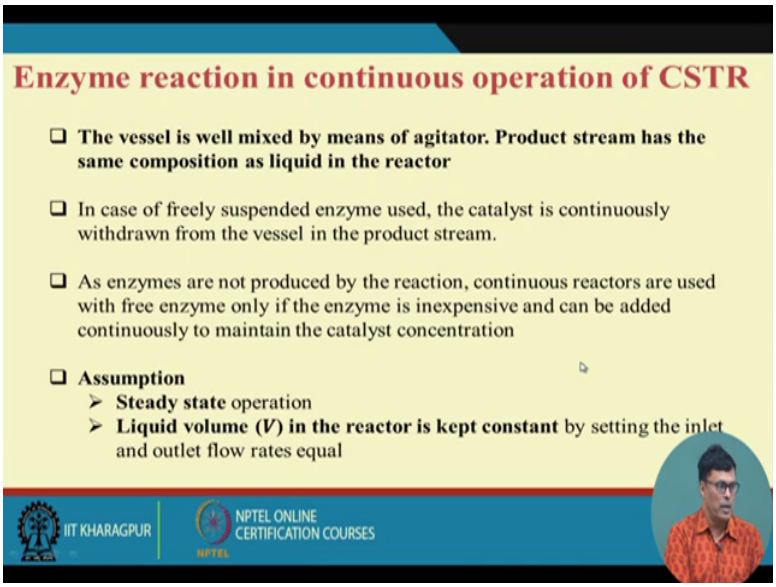

Now next is that an enzyme reaction in a continuous operations CSTR. CSTR we know the continuous stirred tank reactor. The vessel is well big by means of agitator and product stream has has the same composition as liquid in the reactant. Now here I want to find out that that the.

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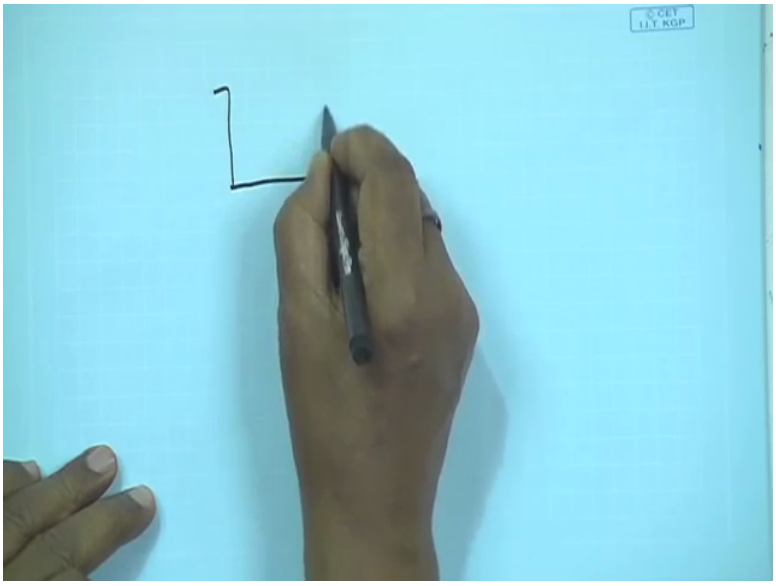

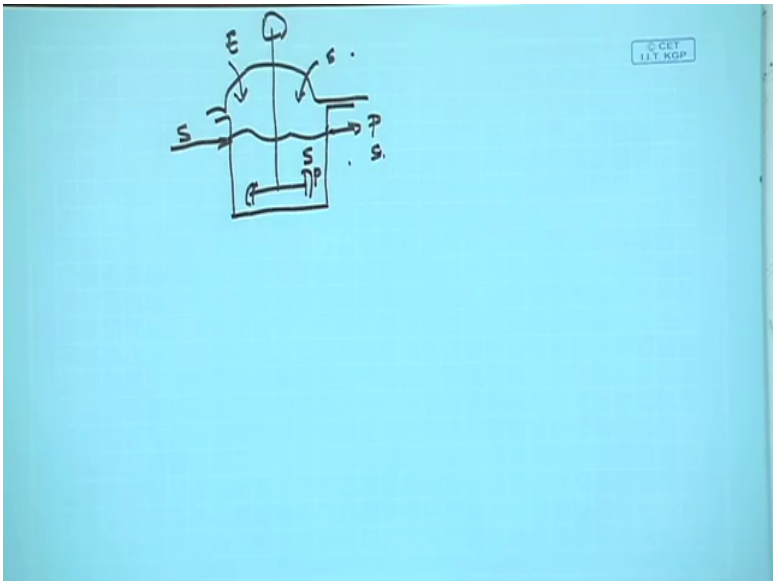

How we operate it the enjoyment rect that CSTR how you operate the CSTR? It like this so initially what you do we put the substrate here we put the enzyme we put the substrate here okay. Then then what will happen that the enzyme substrate that when I usually go when we operate the continues first we want to operate in the Batch Mode let the reaction take place when rate of reaction is maximum.

Then you put your substitute here substrate or enzymes whatever you won't you put it here and take the product. If you operate the system for maybe you can have some kind of membrane here so that the your your your enzyme cannot go out.

So that you know your enzyme concentration in the reactor remain constant so if you if you operate for longer period of time a time will come when this substrate concentration and product concentration here will be same as the other as the (())(22:40) concentrations at the reactor and then and only then it will obtain the steady state condition.

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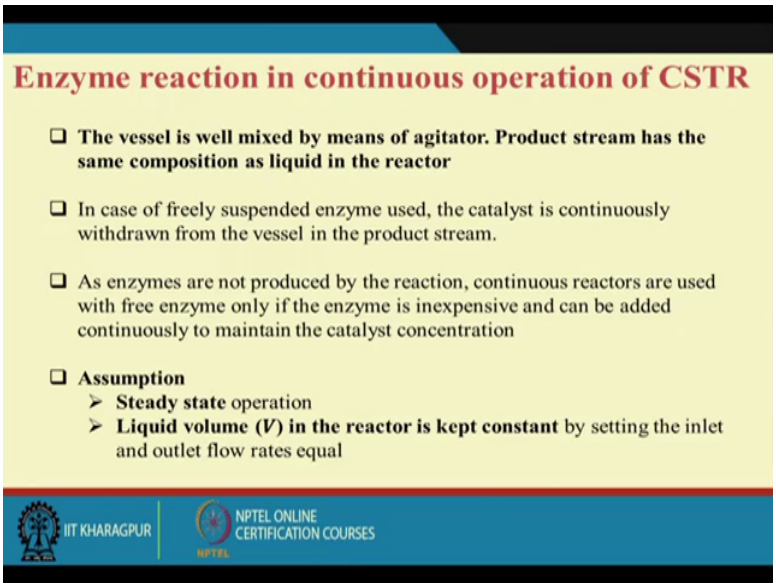

So in the in a particular particular CSTR it is possible to attend the steady state condition at that the infinite period of time in case of freely suspended the catalyst is continuously withdrawn from the vessel that is the major problem major drawback of the CSTR is that continuously your enzyme will be losing that is why I told you that if you use some kind of membrane.

So that you know you are don't allow your your enzyme to go out then then it is possible to maintain the that a constant reaction what if you allow you to go it then whatever enzyme that is going out same amount of enzyme we have to put in the reactor then and only then you can attend the steady state condition otherwise it is very difficult to obtain.

The enzymes are not produced by the reaction and continuous reaction out used with the free enzyme only if the enzyme is in the expensive and can be added continuously to maintain the catalyst concentration constant. So you know that and that is why we use some kind of immobilization technique.

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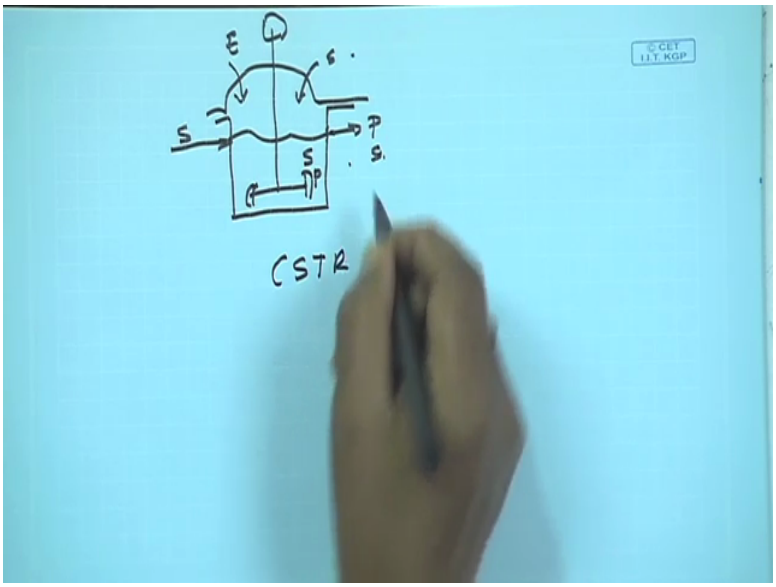

Enzyme reaction in continuous operation of CSTR

- **The vessel is well mixed by means of agitator. Product stream has the same composition as liquid in the reactor**
- In case of freely suspended enzyme used, the catalyst is continuously withdrawn from the vessel in the product stream.
- As enzymes are not produced by the reaction, continuous reactors are used with free enzyme only if the enzyme is inexpensive and can be added continuously to maintain the catalyst concentration
- **Assumption**
  - **Steady state** operation
  - **Liquid volume (*V*) in the reactor is kept constant** by setting the inlet and outlet flow rates equal

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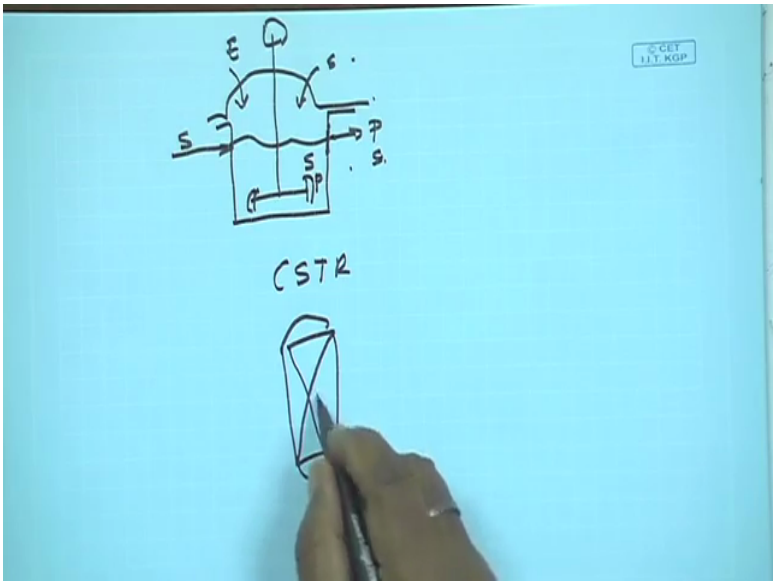

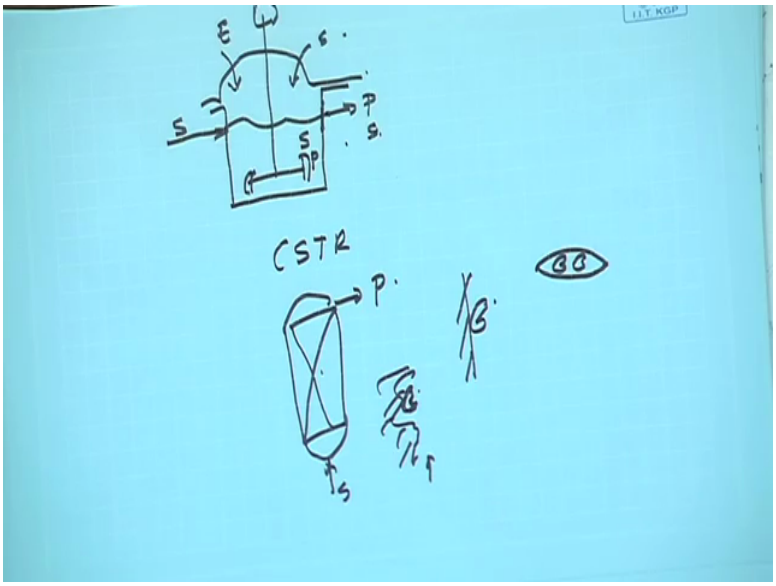

That is supposed we have a column and in this column if we if we fix the enjoying on a solid Matrix and pass here substrate here and get the product and then then there is there is no need of addition of enzyme because the enzymes are there immobilised on the surface of the solid metric This is a solid Matrix the enzyme surface fix on the solid maybe you have some kind of port is there inside the port the enzyme are immobilised.

So when you pass your substrate it give the reaction but it will not go out of the system suppose I told you that collagen membrane we use for putting putting the glucose isomerise enzyme inside and then this bag we put it in the column and then we pass the glucose inside the through this column when it pass.

And glucose enter into the membrane and reacts with the glucose isomerise enzyme and produce fructose fructose then comes out from the membrane and then the ultimately goes out like this so this is how we can do that then in that case we don't have to add enzymes outside.

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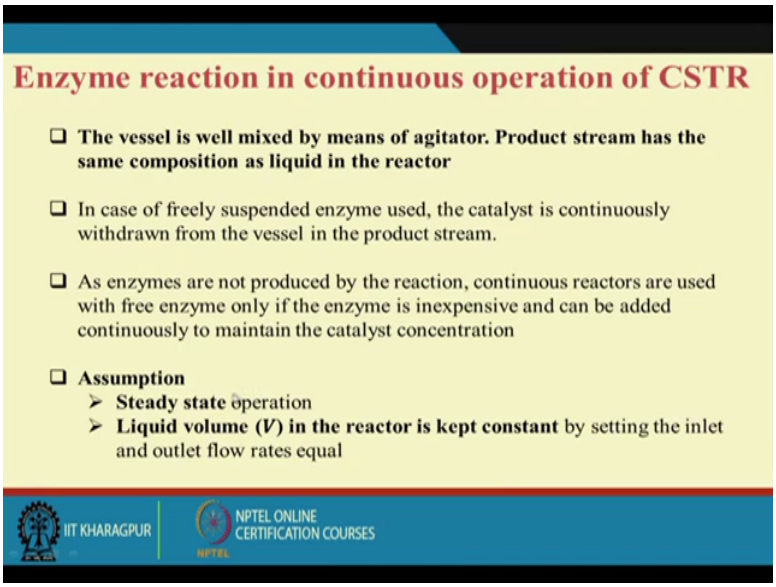

Now assumption is the when you do this analysis of the reactor we assume the steady state operation steady state means when the concentration is constant in different parts of the reactor and liquid volume of the reactor is kept constant is by setting the inlet and outlet flow rate equal.

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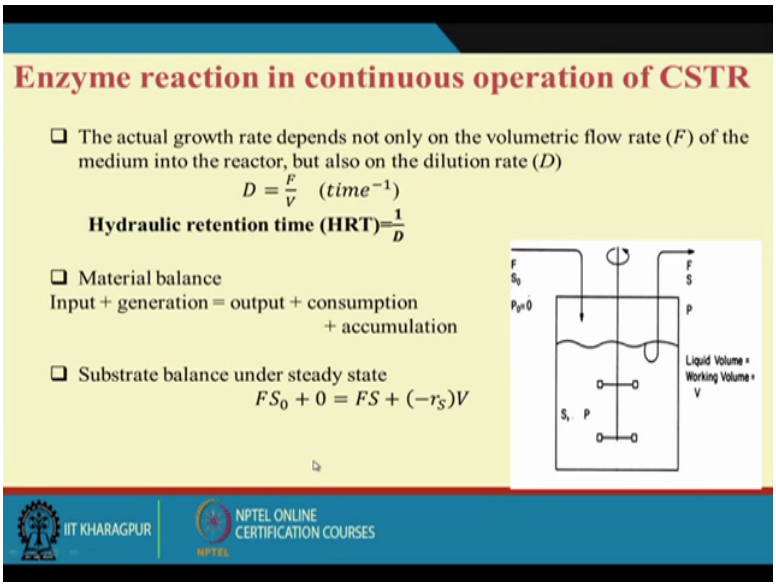

So inlet and outlet few rate this is this flow rate should be constant then and only then is it possible to have the steady state condition now the D equal to this is the dilution rate equal to F by V so dilution rate is directly proportional to the flow rate as to increase the flow rate

dilution rate will be more hydraulic retention time is nothing but 1 by D hydraulic retention time means the how long the liquid resize in the reactor that is called hydraulic retention time

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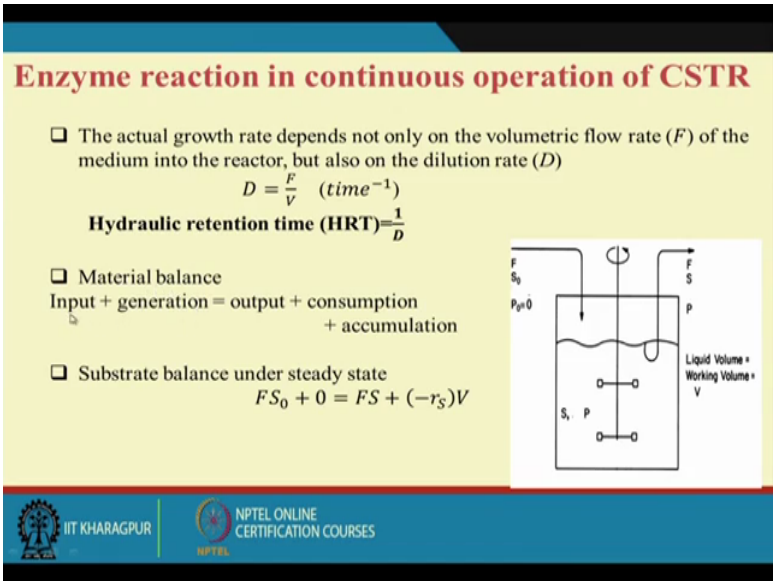

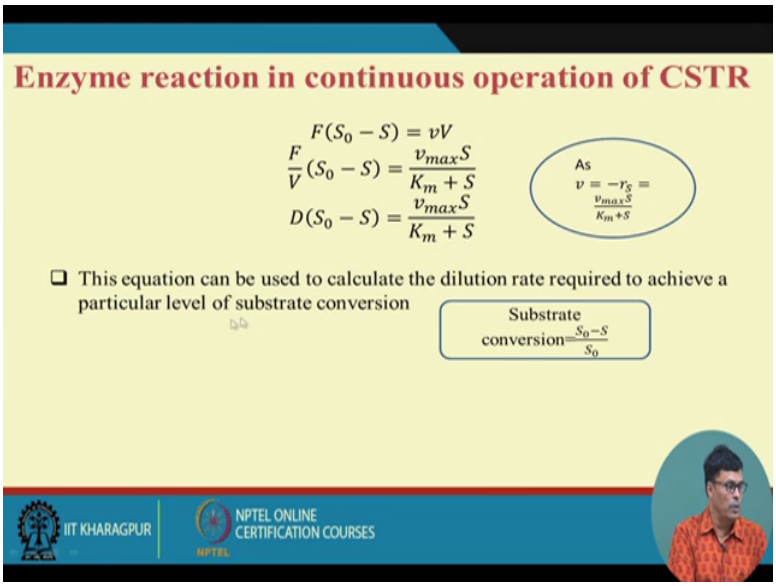

Now for material analysis again the input plus generation equal to output plus consumption accumulation here under steady state condition it is like this F into S zero no generation is there because engine doesn't produce in the system F into S and this the reaction and ultimate this equation is D into S zero minus S Vmax S KM Plus S.

This equation can be used to calculate the dilution rate required to achieve a particular level of concentration conversion so you know if you want to runa continuous system and 1 2 have bill supposed 90% conversion of substrate or 80% conversation of the substrate assuming the

enzyme concentration is constant if you keep the enzyme concentration constant then it is not possible so then only we can we can we can use this delusion rate we can calculate that.

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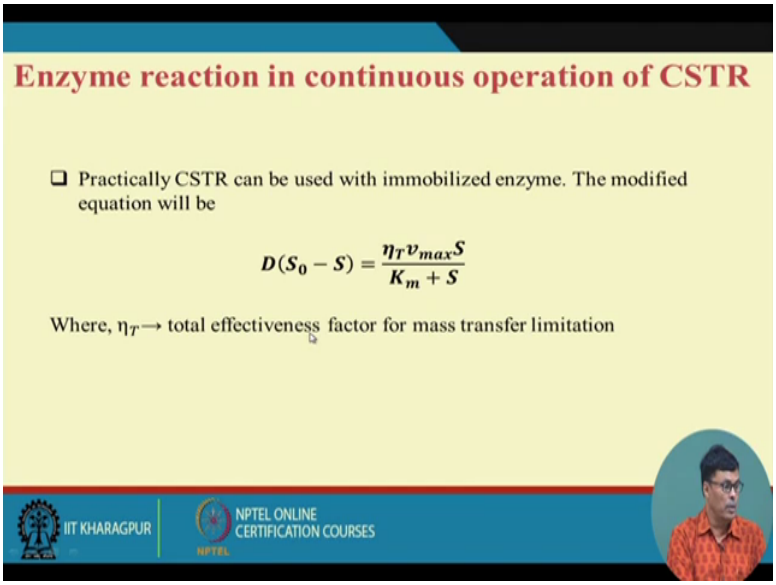

Now when practically that is CSTR can be use with immobilized enzyme the cycle discuss in details after work and this is equal to eeta T that factors is to be eeta T is the effectiveness factor that what the mass transfer limitation as they discuss discuss this separately.

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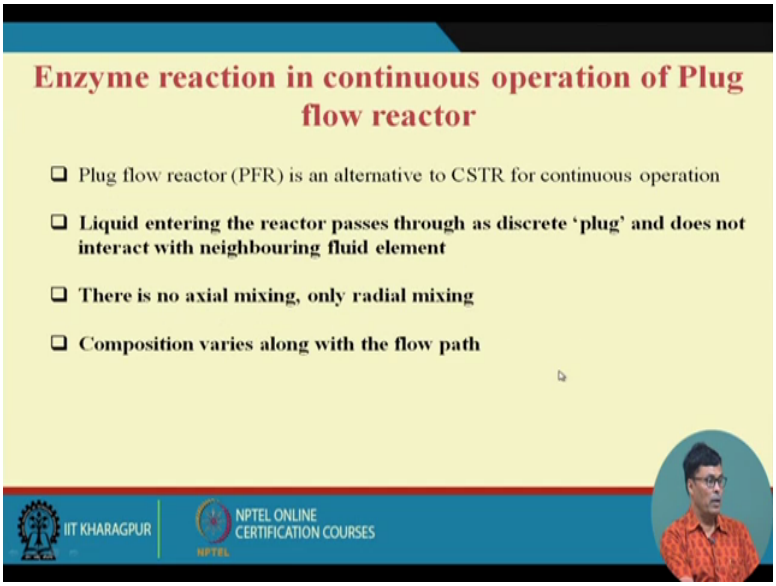

Now enzymes enzyme reaction in continuous operation of plug flow reactor. Plug flow reactor is an alternative to CSTR for continuous operation liquid enter into the reactor passes through as discrete plug and does not interact with the neighbouring fluid element there is no

actual mixing only the radial mixing and composition varies along with the flow path this is the I told you the plug flow beams (())(28:05)I told you that.

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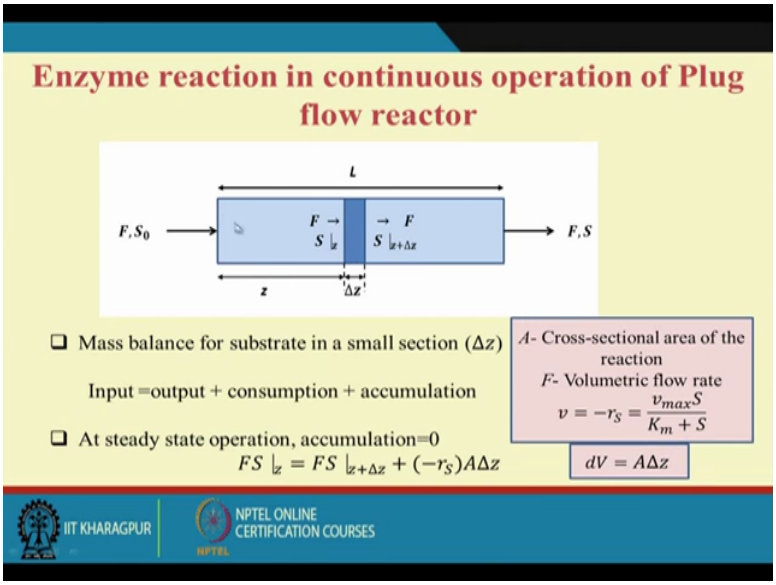

That is there is a thing that is this can be schematically it can be explained like this this is the plug with this is a tubular type of reactor and here we are show me a segment where this is this is length is Z and this is F is flow rate and S is the substrate concentration as Z plus to when it cost Del Z it is S Z plus del Z so steady sate accommodation we can assume to be zero here and then rate of input equal to rate of output plus rate of disappearance that you know that that you can we write this equation like this.

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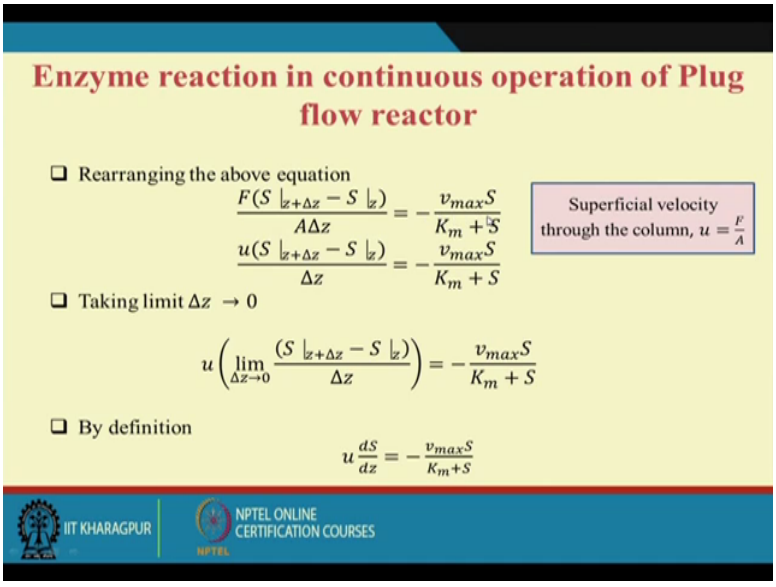

And from that this is equal to this is that you know that this is this is the rate of reaction this can be replaced by the Michaels menten equation it is Michaels menten equation we can write an ultimately we come across this this is U DS by DZ though Vmax by S KM plus S.

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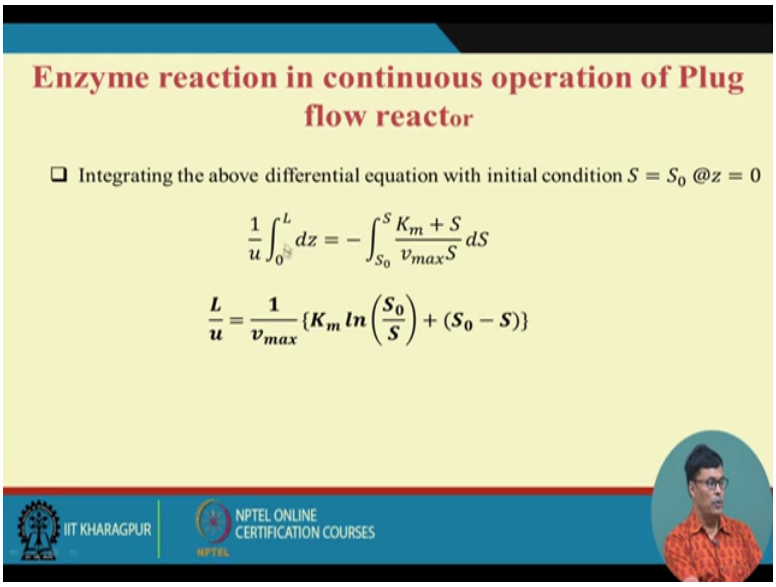

Now DZ is the length that is the 0 to now in this rector we have considered dz now you have to extend from zero to L because the L is this the length of the reactor and then.

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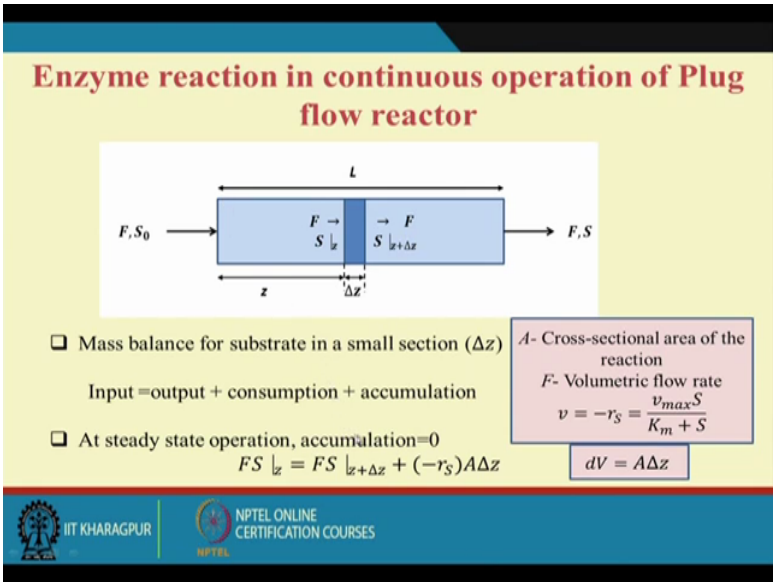

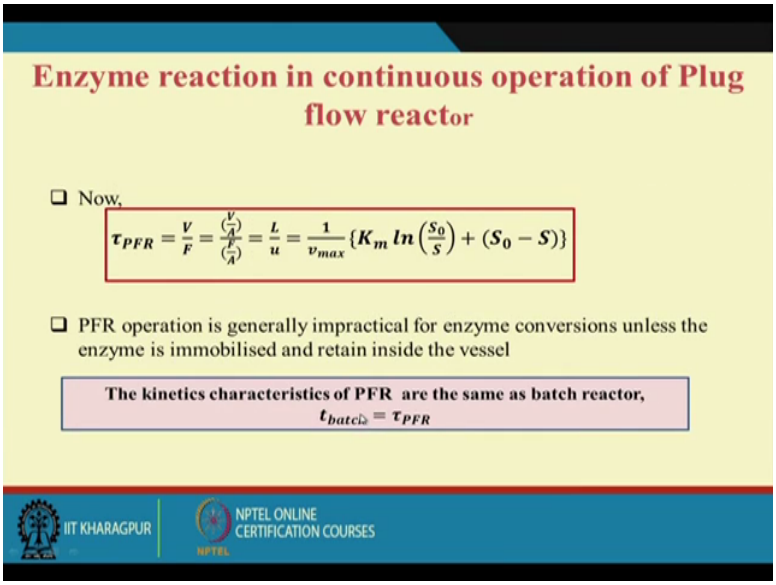

If you if you do this that the 0 to L then it is coming as L by U equal to this this equation and through tau plug flow reactor can be expressed as V by F then L y U, L is the length of the reactor U is the velocity of the fluid then this is divided by this is the called the residence time this is the night in the space time of the plug flow reactor that will be equal to like like this.

So in a plug flow reactor operation is generally in practical for enzyme conversion unless the enzyme is immobilised and retain inside the vessel the kinetic characteristics of plug flow reactor same as the batch reactor. I just showed you that batch reactor the concentration changes with respect to time in the plug flow reactor concentration changes with respect to length of the reactor so that is why the expression for the both the tau plug flow reactor and T batch that is T batch is equal to Tau plug flow reactor. Thank you!