Aspects of Biochemical Engineering
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Lecture - 26
Kinetics of enzyme catalyzed reactions using free enzymes- IV

Welcome to my course Aspects of Biochemical Engineering. In the last lecture I tried to cover (Refer Time: 00:26) problems on these enzymatic reactions. I tried to find out that what is the time required for the conversion of the substrate or how much time required for a different percentage of substrate conversion. Then we tried to find out that what is the effect of inhibitors on the enzymatic reaction, particularly how it is interact with the with the enzymes and how substrate enzyme interaction that differs with respect to inhibitors. And then finally, I also tried to discuss that that how pesticide concentration complex organic molecule like pesticide that how it can be determined with the help of enzymatic reaction.

Now this lecture, this is a very important lecture the reason is that I told you that one of the important aspects of this chemical or biochemical engineering that we at the end we should try to find out for certain conversion of substrate 'what is the volume of the reactor?' Suppose we are walking with a particular industry and industry they are targeting for certain amount of product formation and for getting certain amount of product that what is volume of the of the reactors.

Now, the in one of my lectures I tried to solve similar type of problems with respect to glucose to fructose conversion by with the help of glucose isomerse enzyme. But in this today I want to discuss is special type of problems where substrate is sparingly soluble in water. As you know the enzymes enzymatic reaction usually take place in the aqueous solution. So, (Refer Time: 02:38) your substrate is soluble that enzymes substrate interaction does not take place. So, the solubility; that means, solubility is a very important factor, solubility of the substrate is very important factor. And also that cost of the substrate that was a very important factor, because suppose if the cost of the substrate is very high, so we always expect our conversion of the substrate should be as high as possible.

Now, let us go to the problem.

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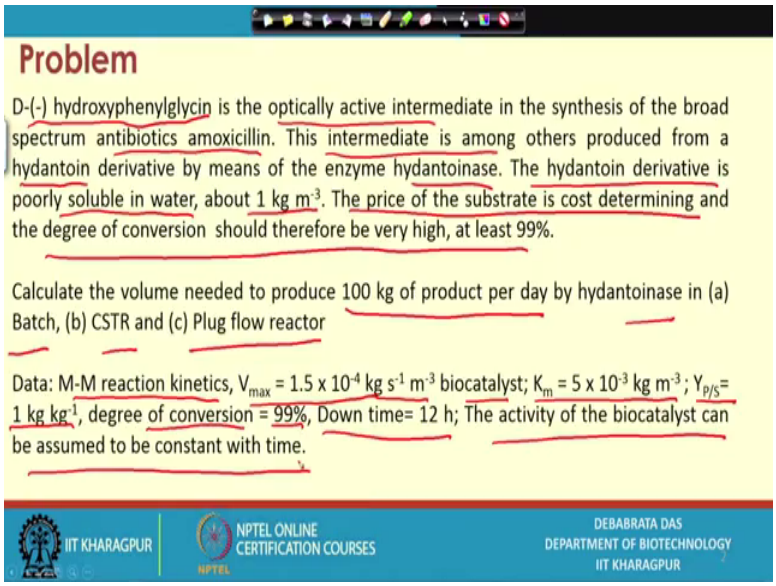

Now the D minus hydroxyphenylglycin this is the complex molecules is optically active intermediates in the synthesis of broad spectrum antibiotics amoxicillin. We use I think day to day life lot of antibiotics, doctor recommends for the curing different type of disease, one antiboiotics will largely used that is the amoxicillin. And this intermediate is among other produced from hydantoin derivative by means of the enzyme hydantoinase. So, hydantoin derivatives that is the name of the compounds substrate, the hydantoin derivative is poorly soluble in water, about 1 kg per cubic meter.

So, there hydantoin derivatives is very poorly soluble in water. As so, the price of the substrate, is cost determining and the degree of conversion should therefore be as high as at least 99 percent. The cost of the substrate is very high, so we expect that the main purpose for this enzymatic reaction to get higher conversion efficiency as high as 99 percent.

To calculate the volume needed to produce 100 hundred kg of product per day; 100 kg of product per day by a hydantoinase in batch process, CSTR and plug-flow reactor. So, three different reactors there you have to find out that batch. And this particular problem also help you for finding out which reactor you will recommend for this particular process, because they know that when you do the calculation you find out that which reactor with the size will be minimum and your design criteria it should be one of the design criteria should be the minimum size of the reactor. Why minimum size of the

reactor, because if the size of the reactor is minimum then cost involvement in the process also will be minimum.

Now, different kinetic constants are given, like Michaelis Menten kinetics, max is 1.5 into 10 to the power minus 4 kg per second per cubic meter biocatalyst, k m value is given Y p by s: Y p by s means p substitute and the product by substrate; that means, the stoichiometry. This indicate the stoichiometry of the process; that means, 1 kg of substrate produce 1 kg of product. And degree of conversion as we mentionedabout this is 99 percent, and downtime is 12 hours. The activity of the bio catalysts can be assumed to be constant with time.

Because, if the activity of the biocatalyst is different with respect to time; differ with respect to time then it is very difficult then we shall have to do the integration of that I just show you in the next problem how we can solve that.

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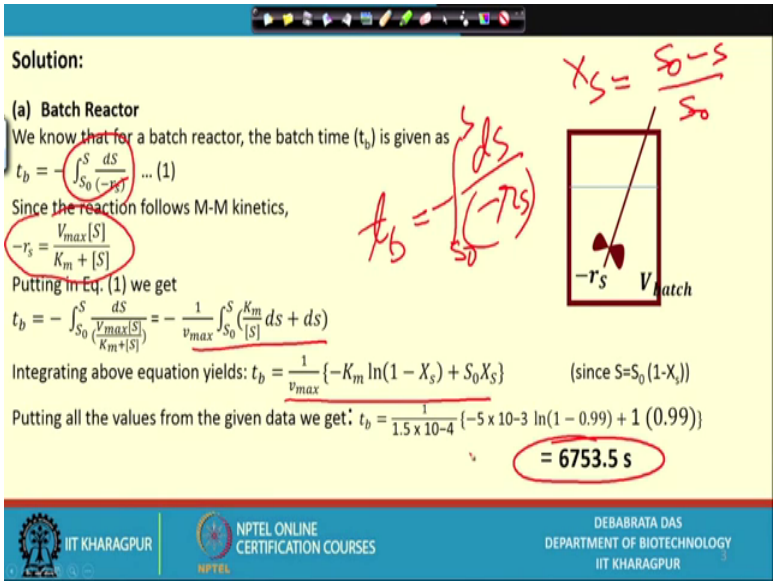

So, for the batch process so what we can do; we know that what is the batch process that we have already know that t b equal to minus ds by minus r s, ok. So, this is exactly what we have written here and this is integrating is 0 to s. Now this is the found Michaelis Menten equation minus r is equal to this equation then we can put these values, then we can get this get this the equation that t b equal to minus 1 by V max is 0 to s. This came by ds by s plus ds.

Now this can be converted in this form this is the X s. What is X s? X is equal to S0 minus S by S 0 this is they say X s s. So, if you put this value will find the time required for this batch process is coming about 6753.5 seconds.

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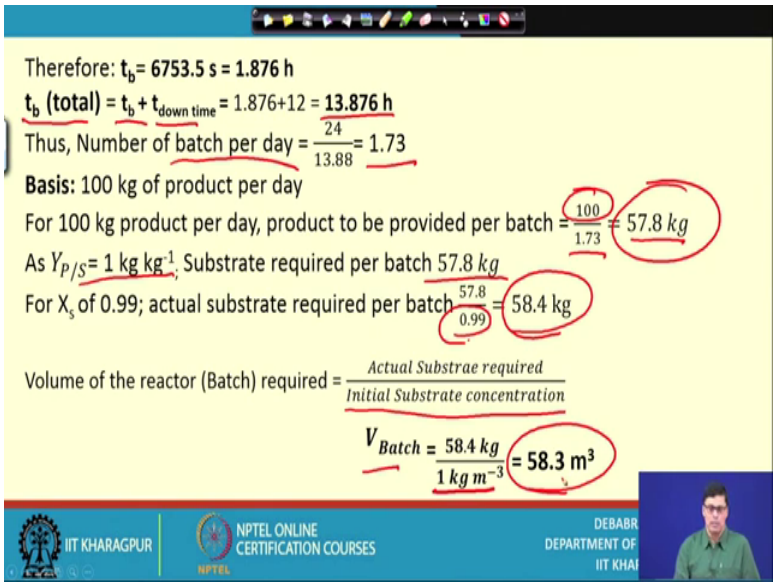

Now, this is a we can convert into hour. This is the equivalent to 1.876, am I right. Now I told you can remember that when we want to determine the volume of the batch process first we select to find out how much product you have to produce per batch. So, I can show you again.

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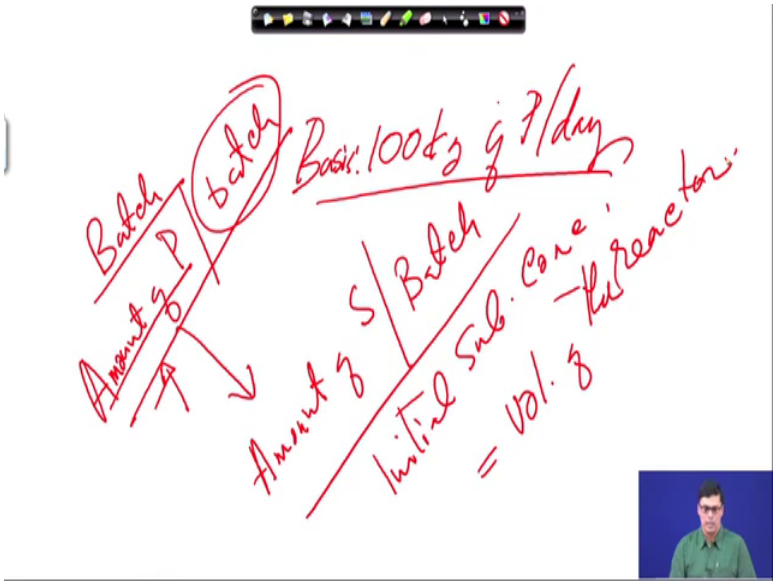

So, what I told in case of batch process, when you find a volume of the batch first we shall have to find out amount of product to be produced per batch. So, but when you to when in this problem we target maybe 10 kg of product, the basis what is the basis 10 kg of product per day let us assume that ok. Now when you try to find out the batch volume we shall have to find out to get this much of product how many batch you have to operate per day, because if you know the number of batches to be operated per day from that we can find out how much product is to be produced per day.

Now if you know how much product is produced per day, then from the stoichiometry you can find out amount of amount of substrate that required per batch. Am I right? Now once you know amount of substrate required part batch. Then if you divide by initial substrate concentration then what you will get, you will get the volume of the reactor very simple. Very simple you can find out the volume of the reactor, am I right? So, let us see how we can solve this problem.

Now, here the total time is coming about 13.876, this is the batch time plus downtime. I told you in case of batch process there is a time downtime you have to consider, what is downtime? Down time include the time required to take out the material after the reaction is over and then you clean the vessel and also refill the vessel, because this downtime is very much (Refer Time: 10:50) if we considered as the ideal time because during that time no reaction take place.

So, this when you try to find out the volume of the reactor then we shall have to find out that we shall have to add this down time with the batch time. So, total time that required in the batch process is will be coming about 13.876 hours. The number of batch per day is about 1.773 Am I right?

Now, how much product we shall have to produce? 100 kg. So, if you divide by 1.73 so, how much product you have to produce per batch: 57.8kg of product per day batch, am I right? Now we have already find out from the stoichiometry 1 kg of substrate can give 1 kg of product now if it is like this we can easily say that for the getting this much of product that we require the same amount of substrate that is 57.8kg of substrate. But in this problem in that conversion efficiency has been given 99 percent, am I right? So, this is to be divided by 0.99. So, actual amount of substrate required that will be 58.4 kg.

Now, I told you the volume of the reactor then what will be equal. Now initial substrate concentration in this problem the initial substrate concentration is not given, but certain criteria is given that the since your substrate is very costly and then they are they are saying that this is the substrate is the that is the limiting factor. So, what we assume the maximum (Refer Time: 12:40) possible substrate concentration to get the maximum amount of product. Thus, what will be the maximum amount of substrate concentration that will be the solubility of the substrate; whatever solubility we have and solubility of the substrate in the water is about 1 kg per cubic meter.

So, if it is like this. So, I can assume the initial substrate concentration is 1 kg per cubic meter. So, you this if you divide by this you will get the volume of the batch process. This is how you can easily find out volume of the batch process.

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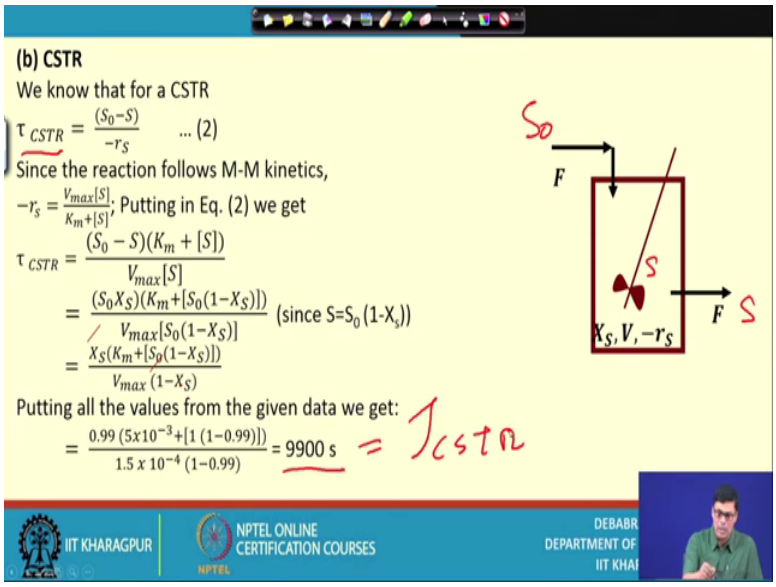

Now, let us see how you can find out the volume of CSTR. Now in case of volume of CSTR the tau CSTR tau CSTR is nothing but the space time of the continuous start time reactor s 0 if the initial substrate concentration minus this minus r s. So, this is like this, here is the S0 and this is the S here is. So, here at the under steady state condition here also S, am I right? And then we can write this equation is in this form and finally, we can get this particular expression. And then we put the value of X s that 90 x s is 99 percent conversion and then we find out the tau CSTR, this is equal to CSTR value is this. And once you know tau CSTR value then we can convert the in terms of hour. Am I right?

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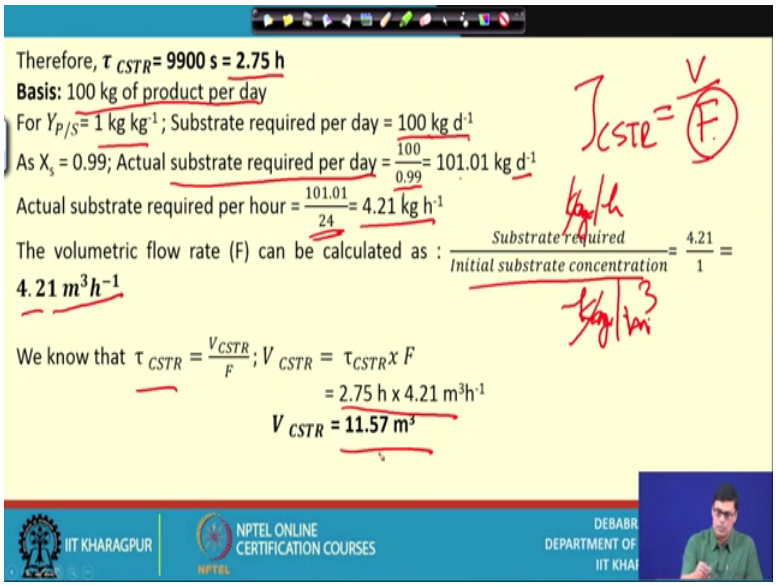

Now in this problem that one thing we know tau CSTR is equal to what equal to V by F, and what is F? F is the volumetric flow rate. Now here we shall have to find out the volumetric flow rate. How we can find out, how much substrate you are using per day 100 bigger 100 kg of product per day. Now for 100 kg of product how much substrate is required 100 kg per day same amount of substrate because this is 1 kg of substrate product produced from 1 kg of substrate; the actual amount of substrate required per day will be what 100 divided by 0.99 that is 101.01 kg per day. Am I right?

Now, actual amount of substrate required that this is in day we can convert with respect to hour if you divide by 24. Then, because one day equal to 24 hours so it is coming about 4.21 kg per hour. Now this substrate concentration, when we divide by for the initial substrate concentration of substrate. nNow this unit is kg per hour, am I right? And this is kg per hour and this is kg; this is initial concentration is kg per unit per cubic meter am I right.

So kg will cancel. So, this will be becoming cubic meter per hour that this is coming out our 4.21 cubic meter per hour. Then now the tau CSTR already we find out 2.75 and if you multiplied by, so V will be equal to tau CSTR into F. So, if you would like this we multiply we find out the volume of CSTR this the 11.57 cubic meter.

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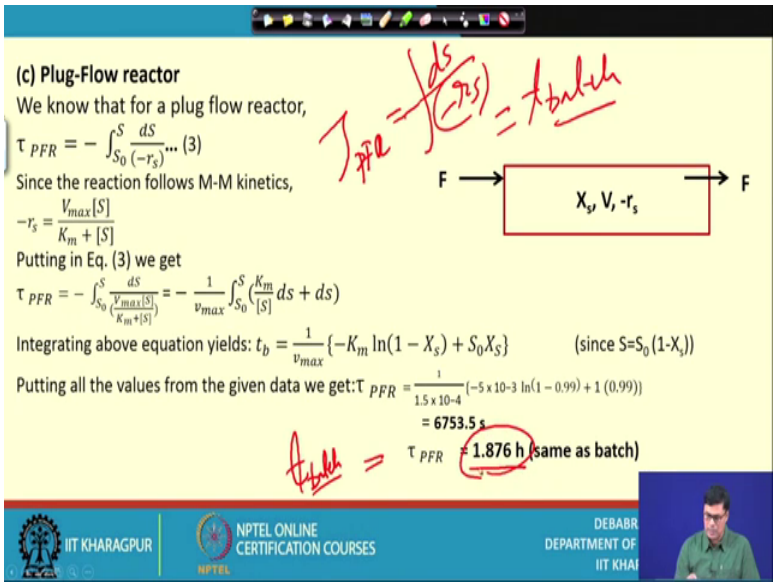

Now, let us see in case of plug-flow reactor. Plug-flow reactor we can similarly find out the same that this tau plug-flow reactor will be same as t batch, because we know that their expression is same. So, both tau plug-flow reactor; what is the expression we have minus ds by minus r s, am I right? This is equal to (Refer Time: 17:05) t batch. So, since it is like this so for the same 99 percent conversion we have the value is same, the time requirement will be the same.

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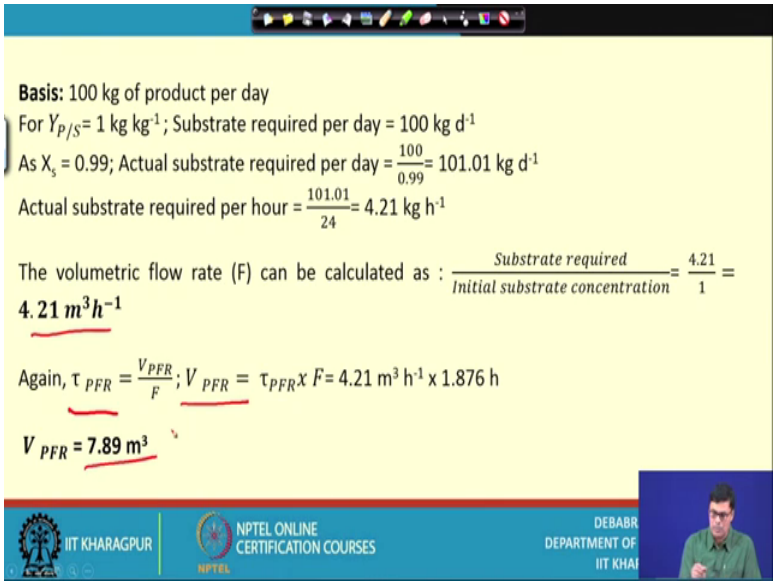

Now, from this we already find out the flow rate in the previous problem. We already find out the flow rate and tau plug flow reactors with the volume by flow rate. So, if it then volume of plug flow reactor is there we can easily calculate like this 7.89.

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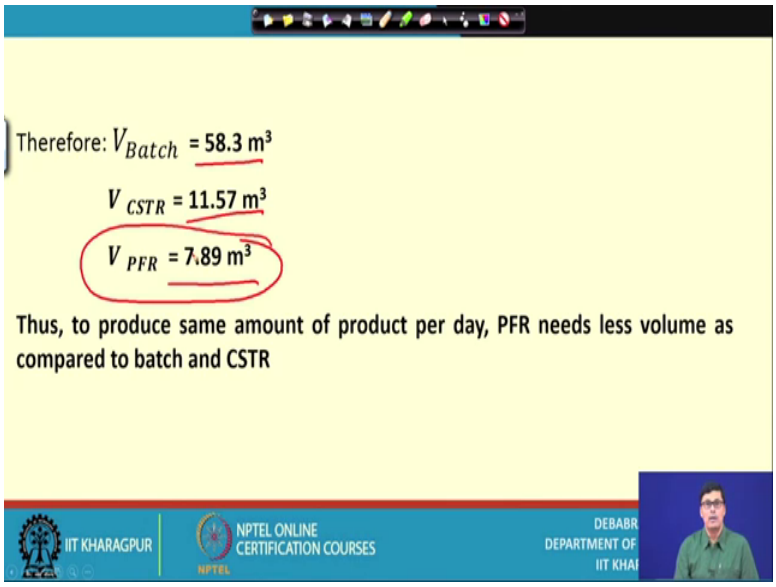

So, in this problem we try to find out the batch process volume is 59.3 cubic meter and CSTR 11.57 and plug-flow reactor 7.89, that means recommendation is the plug-flow reactors, that plug-flow reactor will be most suitable for this particular production process.

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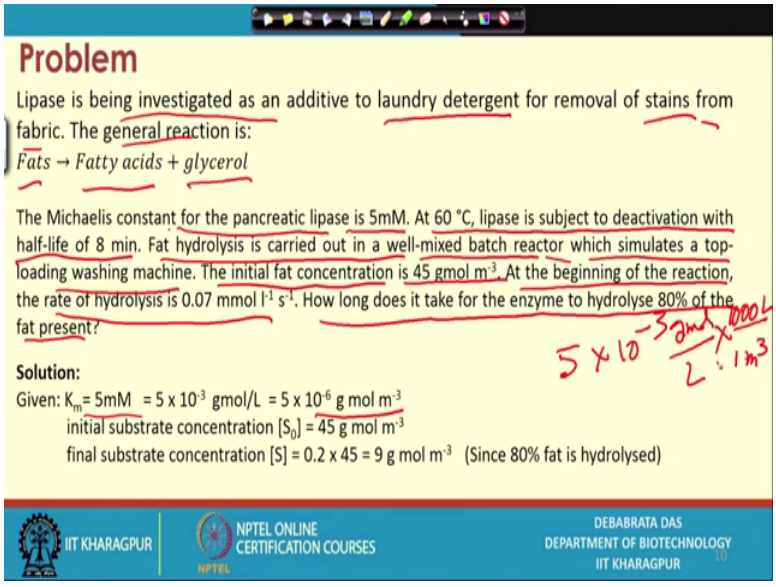

Now, let me go to the other problem because the other very interesting problem that we have that is regarding, we know that enzyme has lot of application in the detergent industry; particularly or the removal of stain that we have in the in our cloth that can be removed with the help of the enzymes. I can give the example that if we have the blood stain that we have in the in the cloth, then we use some protease enzyme that can remove the blood stain. Now this problem is something different, because this problem we are we will be dealing with lipase because due to presence of some kind of lipid or fat or sometimes your cloth also will be giving some kind of stain. So let us see how we can solve this problem.

The lipase is being investigated as the additive (Refer Time: 19:03) laundry detergent for the removal of stains from the fabrics. So, the general reaction is that the fats when in presence of lipase it produces fatty acid and glycerol. So, this is the easter I know that you know you all know that fats is nothing, but esters of alcohol and the and the and the fatty acid. The Michaelis Menten constant for the pancreatic lipase is 5 milli molar. As 60 degree centigrade lipase is subjected to deactivation. I told you that you know that deactivation is a very important characteristics of the enzyme that you know with a half life of 8 minutes. I already discussed; what is half life half life means time required for reducing the half of the activity of the enzymes.

The fat hydrolysis is carried out in well mixed batch reactor will simulate a top loading washing machine. The initial fatconcentration is 45 gram moles per cubic meter at the beginning of the reaction the rate of hydrolysis is 0.07 millimoles per liter per second how long does it take for the enzyme to hydrolyze 80 percent of the fat presence. So, this we said after; that means, when you talk about the removal of stain; that means, how long we should soak the cloth in that particular detergent, so that the you stain can be removed. Now different this value so, because I told you before also when you try to solve any kind of problem I request to all of you please write down what is the data is given.

The k m value is given here, so this can be we can convert it in the gram mole per cubic meter. That we can convert in this unit. And then this conversion I hope you understand that that 5 into 10 to the power minus 3 gram mole per liter, am I right? The how you convert I told you if you want to convert in the cubic meters. So, you write 1 cubic

meter, am I right? 1 cubic meter is how much 1 cubic meter equal to the powered cubic meter. So, you have to find out this is 1000 liters, am I right?

So, anyhow that; so the concentration is coming about 5 into 10 to the power minus 6 gram moles per cubic meter, and S0 is 45 gram mole per cubic meter, and 80 percent substrate is converted so, 20 percent is remaining s o 0.2 into 45 it is coming about 9 gram mole per cubic meter.

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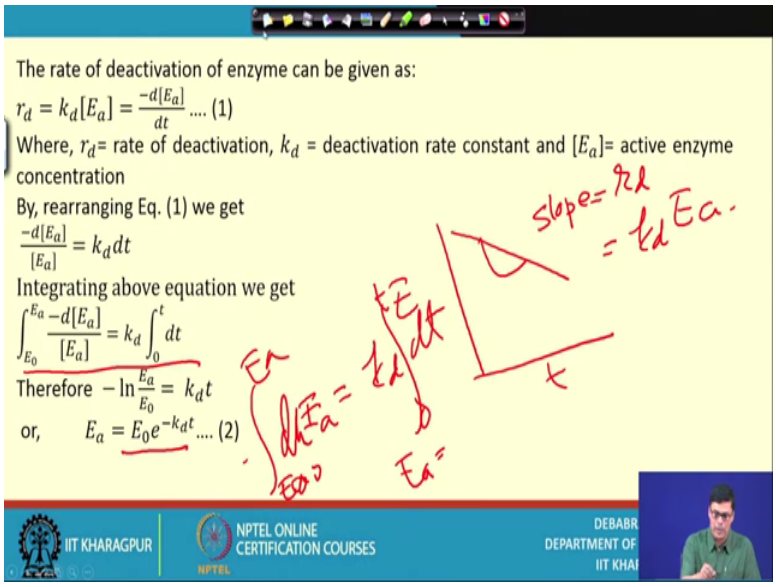

Now, the rate of deactivation that because this is like this that you deactivation when you discuss that is the activity of the enzyme that is decreases like this, am I right or so that is enzyme activity with respect to time this slope. This is equal to this is r d actually, because this is equal to k d into a the enzyme concentration. This is how we have find out this is rd equal to this one. And so enzyme deactivation will take place like this. This is the dt and if we integrate this you will get this kind of equation. And this is d ln d ln E a this ln E a equal to k d into dt am I right.

Now if you integrate 0 to t and this is E0 to E a 0 to e then what will get Ea equal to you can this is the ln E a by a the E a will come E a 0 into e to the power k d into t this minus sign will come here, ok. So, you can easily find out that.

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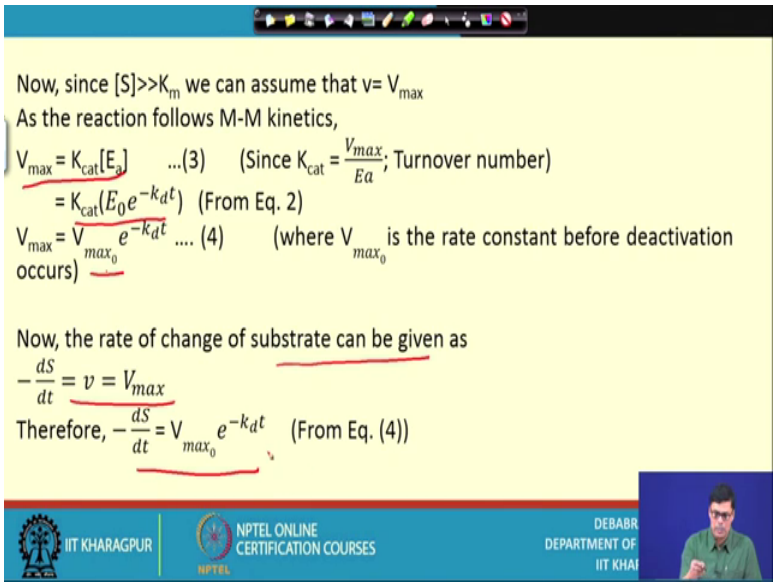

Now, another thing is very important that is V max is (Refer Time: 25:08) related with K cat like this K cat into E a, that now Ea is already we determined this equal to E a 0 into e to the power k dt k one d t. Then this is equal to V max equal to V max 0 e to the power k d t, so we consider 0 stands for the initial substrate concentration. Now the rate of change of the substrate concentration can be given like this then this we can represent it in the form of this, because the initial substrate concentration we can write like this.

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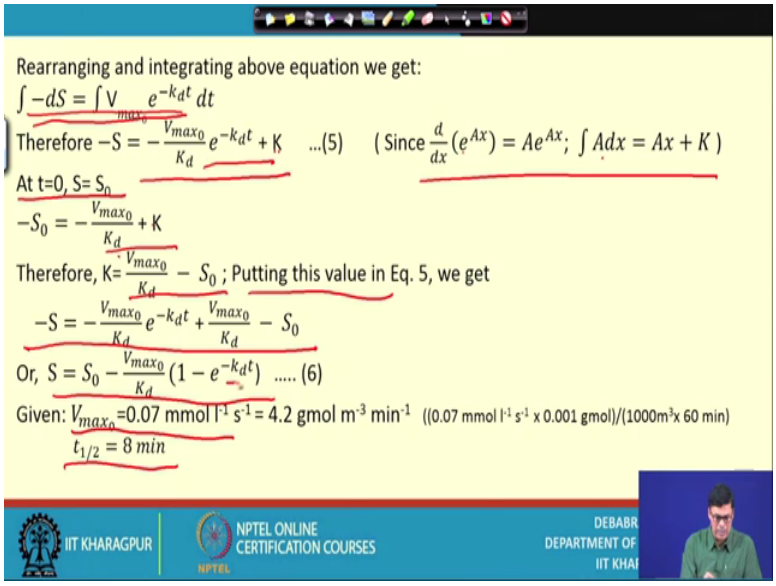

Now, if we integrate that value in that indicate that particular value that minus ds is the V max e to the power minus k d into d dt and this equation is coming like this and we know that this is the kind of integration differentiation that we have d by dx e to the power Ax equal to A e A x this will be A into dx, this is equal to A x plus k. If we use the same thing apply here this equation we can solve in this form.

And then with this is the boundary condition we have at t equal to 0 s is equal to 0 then we can put this equation like this 0 equal to this. And therefore, K will be equal to K I can take this side and this equal to like this. Now putting this value in this equation in the K equation here then we can get this final equation is there. So, this equation we can modify in this form. Now in this given problem the V max is 0 value is given and t half value is given and k d value we shall have to find out and so that we can find out the how much substrate is (Refer Time: 27:09)

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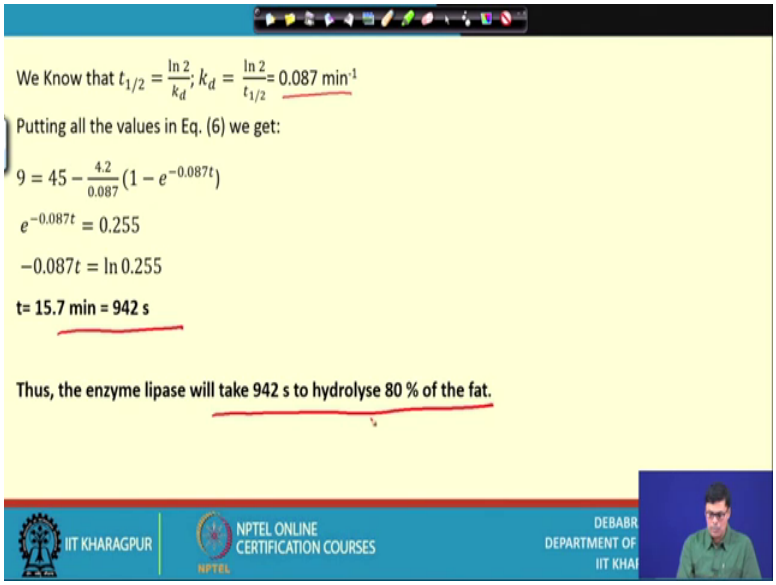

So, t half equal to equal to t 2 into this we know that t 2 into k d the k d will be equal to what this is this value. And then if I previous equation if we put all these values we can find out time required for this particular reaction that we 80 percent the fat degradation. So, our answer to this problem is that is required 942 seconds times that, for the hydrolysis of 80 percent of fat.

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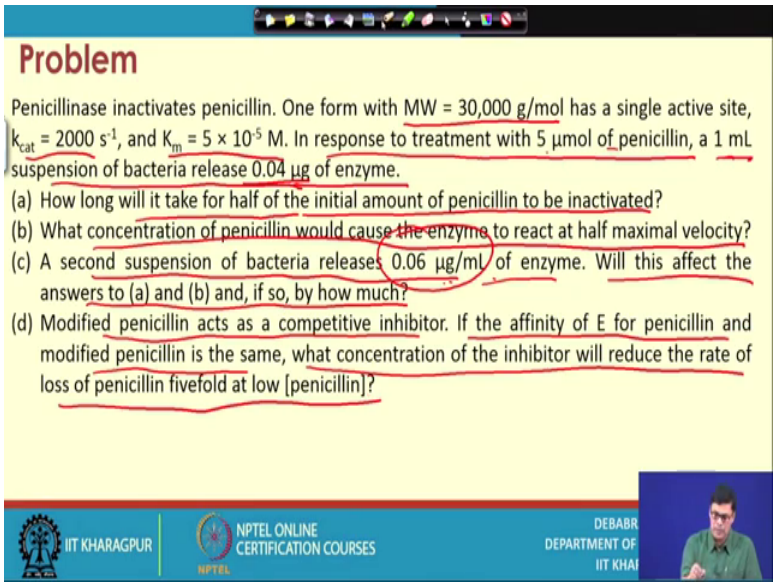

Now another problem similar problem we have that penicillinase inactivates the penicillin and one form the molecular weight is 30,000 gram per mole has the initial active side and k cat value is given, k m values given.In response to the treatment of 5 milli molar penicillin 1 milliliter suspension of the bacteria release about 0.04 micro gram of enzyme. How long will it takes the half of the initial amount of penicillin to be inactivated? And, that means you have to find out, that what is the time required to remove the half of the penicillin and to degrade the half of the penicillin that is we shall have to find out. And initial final penicillin concentration was 5 milli molar that.

Then what is the concentration of penicillin cause the enzyme to react half maximum velocity. Then the second suspension of bacteria release 0.06 micro moles per milliliter of enzyme will this affect the answer a b and if so, high how. Now initially that it was the 0.04 milli micro gram, now it is 0.06 microgram if it is released like this that will is going to happen this is also per milliliter because 1 milliliter of self suspension is there. And modified the penicillin acts as a competitive inhibitor, if the affinity of enzyme for penicillin and modified penicillin is the same what concentration of inhibitor reduce the rate of loss of penicillin 5 fold low of penicillin.

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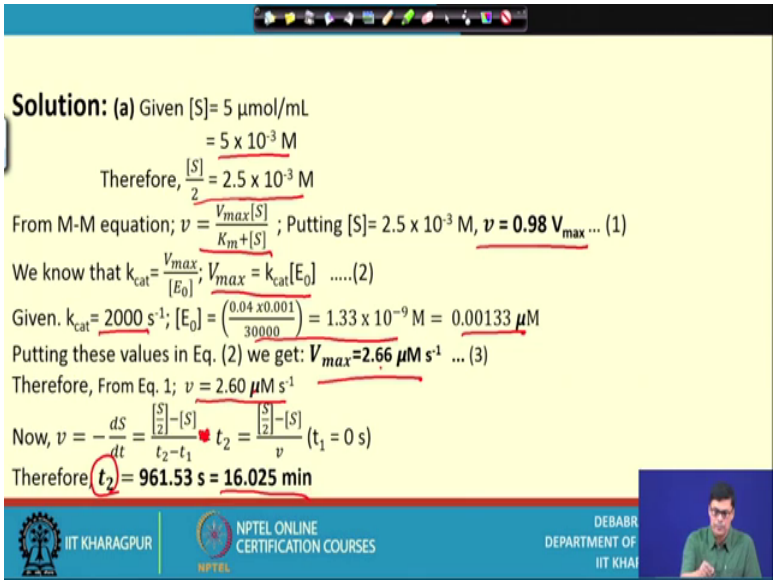

So, you know this is the problem that we have. Now we shall have to, let us see how we can solve it. I hope this solution is given, I hope you can easily understand how it is solved, and initial problem is the how to what is the time required to degrade half of the penicillin the initial penicillin concentration is this micro moles.

So, this is s by 2 is this and velocity of reaction is this so K cat K cat value is the given there is 0 value from the K cat value we can find out the E 0 value because V max equal to K cat K cat it into is 0. So, we can find out from that we can find out V max value. Once we have this we can easily find out that the v value is this from the equation one: we can find out this v is equal to 0.98 into V max we put this V max value here and we find the v value. Once you have the v value we have the up the degradation how much time is the velocity is equal to this is equal to we can find out this is t minus t 2. So, this is not there. So, t 2 we can calculate this is divide by v. So, then t 2 will be equal to 16.025 minutes.

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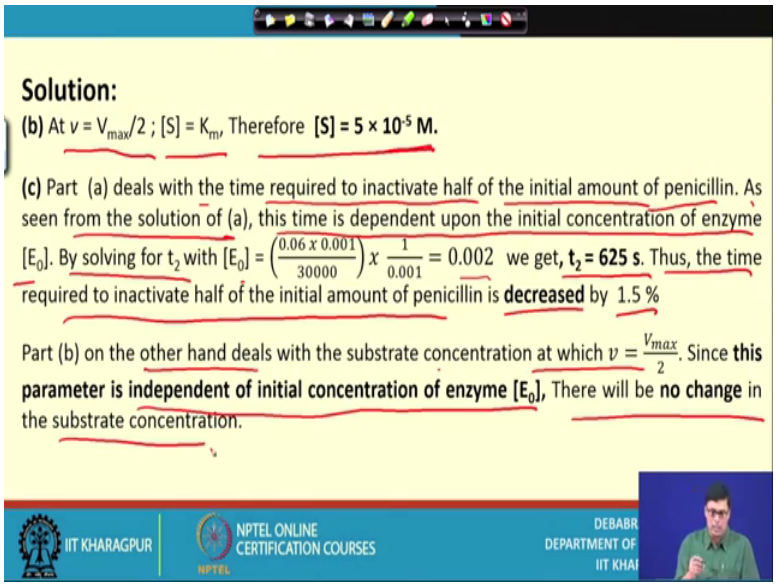

Now, next problem that, next solution that we have at the v equal to V max by 2 is the equal to equal to K m. Therefore, with that we can easily find out because max value already we know. So, we can easily find out that s value find out, so they can find out what is the S value that K m value from the K m value we can find out s value.

And part a deals with the time required for inactive half of the initial amount of penicillin as seen in this solution one this time is depend upon the initial concentration of enzyme is 0. By solving t 2 is 0 equal to this we get t 2 is this, then thus the time required for inactive half of the amount is decreased by and decreased by 1.5. So, if that question was that if you increase the enzyme concentration how we are going to affect this system. And then part other than substrate the velocity at which v equal to since this parameter is the independent of the initial substrate concentration initial concentration 0 there is no change in the substrate concentration.

So, this is the typical problem that we have.

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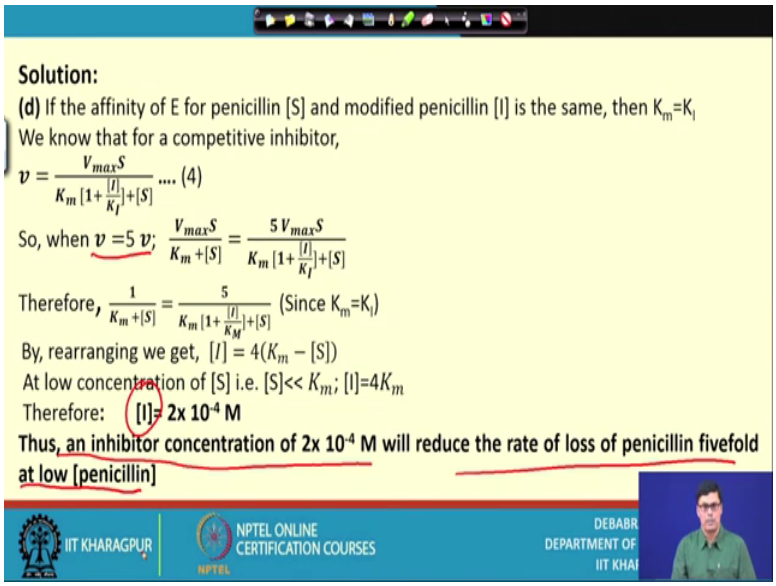

And this is the then finally, we can find out the inhibitor concentration I am not going in details. So, I hope form this equation same as we have done before it is similarly solved it. So, I hope you can understand that, this is a 5 time this velocity if you increase that what will be that that 5 time so, form that you can find out that; so then final conclusion is that an inhibitor concentration 2 into 10 to the power minus 4 will reduce the rate of loss of penicillin by 5 folds at low penicillin. So, no that is that is kind of conclusion that we have from these particular problems.

So, in conclusion of what I want to tell that in this particular lecture I try to discuss that how you can find out the volume of different type of reactor batch CSTR and plug-flow particularly in case of sparingly soluble substrate. And also I try to discuss other different aspects that how that lypase that you know which is very much used in the detergent industry, how it is activated, because how much time is required for removal of the fat that you know that how we can calculate. And finally, we are talking about the penicillin deactivation of penicillin with the help of penicillinase enzyme how it affects that activity of the penicillin.

So, I think it will be better if you practice this problem, because this required lot of practice, where and if you have any kind of problem I am very happy to answer it.

Thank you.