Aspects of Biochemical Engineering
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Lecture – 40
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – X

Welcome back to my course Aspects of Biochemical engineering. Now I was discussing about the kinetic of substrate utilization, product formation, biomass, production using microorganism.

So, we discussed the theory part as well as we discussed several problems and coming two lectures also we will try to solve to more solve problems because what I believe that? If we can solve more problem the conception of the on the microbial process would be mode; how to analyze the chemostat? How to analyze the plug flow reactor and how to analyze the fed batch process?

Now, so, today this whatever lecture we have we have frame that we will try to go much details of this particular microbial fermentation process the first problem that I want to discuss the.

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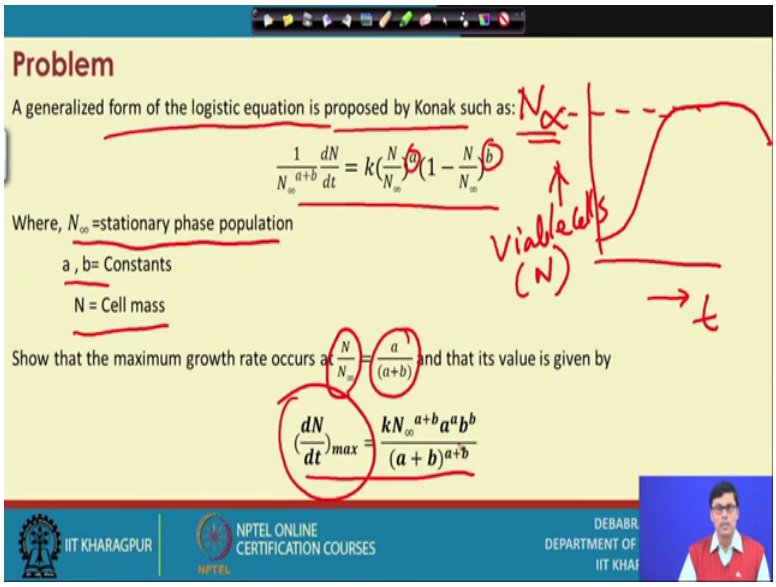

If you look at this problem this is a generalized form of the logistic equation is proposed by Konak such as this is the equation.

Though. So, we know if we if we can remember the life cycle. So, it is like this time with respect to viable cells am I right viable cells. Now these viable cells we can express in terms of N. So, it is like this it is going then station it then coming back. So, here here we consi this is we will considered in all N infinity or in then infinity means the cell mass number of cell mass at the stationary phase.

So, this is the in infinity the stationary phase population a b is the constant this a and b at the constant and N is the cell mass concentration at any time t. Now what we shall have to do? So, that the maximum growth rate occurred; when the N by N all infinity equal to a by a plus b and that it is value is given by this equation. So, if you put these values in this equation that we can find out the maximum rate of that cell mass production that should be equal to k into N infinity a plus b a to the power a b to the power b divided by a plus b to the power a plus b.

So, this is a very interesting problem now to solve this problem what I want to tell.

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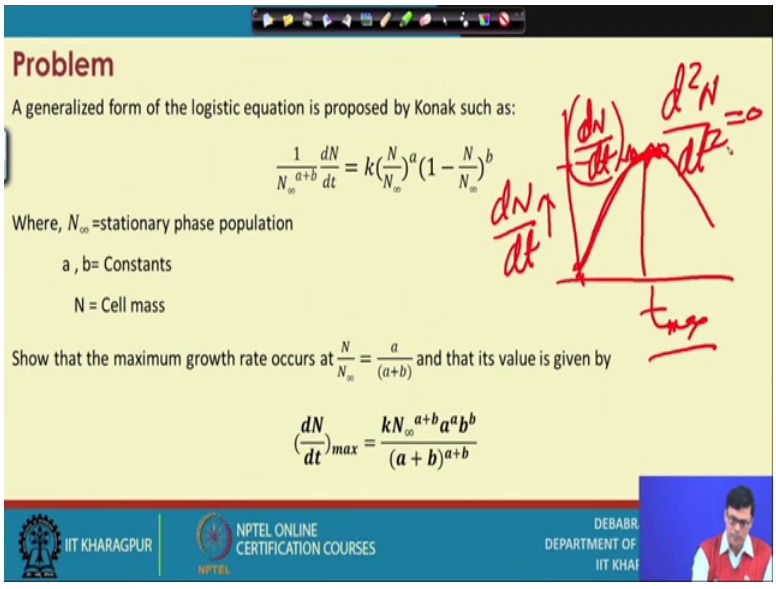

That we know that dN by dt what is dN by dt rate of cell mass formation this is with respect to time. Now, what will happen this will keep on increasing with respect to time. Then we will decrease now this we shall have to monitored this is this is what is this

point? This point is dN by dt this should be maximum. So, the this is also t max. So, at t max we will get the rate of rate of cell mass formation is maximum when it will occur when D 2 N by dt 2 this should be equal to 0 because it increases then I that in the Plato when it is at in the Plato then it will be maximum and this is possible when D 2 by D 2 N by dt 2 equal to 0.

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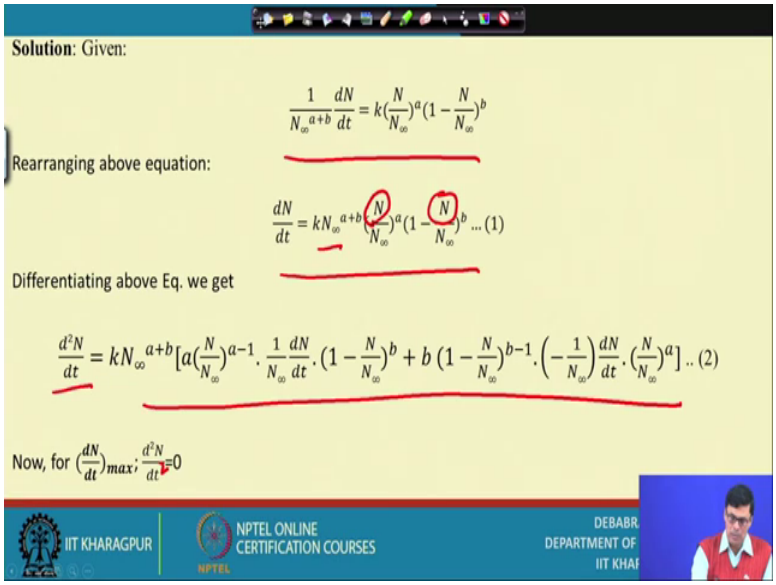

Now, this is the solution how we try to walk out with this problem. If you look at here that this is the problem that has been given now if I take N infinitive a plus b and the right hand side; the equation will be becoming this is the equation we will get like this and then if we differentiate with respect to time then with their by differentiation we will get this equation.

. So, and then after getting this equation; then we put this conditions and when dN by dt equal to max the dt 2 by dt dt dt N dt 2 N by dt 2 this is a mistake is there you can correct it this should be equal to 0.

So, this I can I can I can explain you little bit to you that; you know that this if you differentiate this that you know that this is in infi this is constant am I right, but this is N is the variables and this is also variable. So, we differentiate with respect to this two term and when we differentiate with respect to time this will this will be this will come like this.

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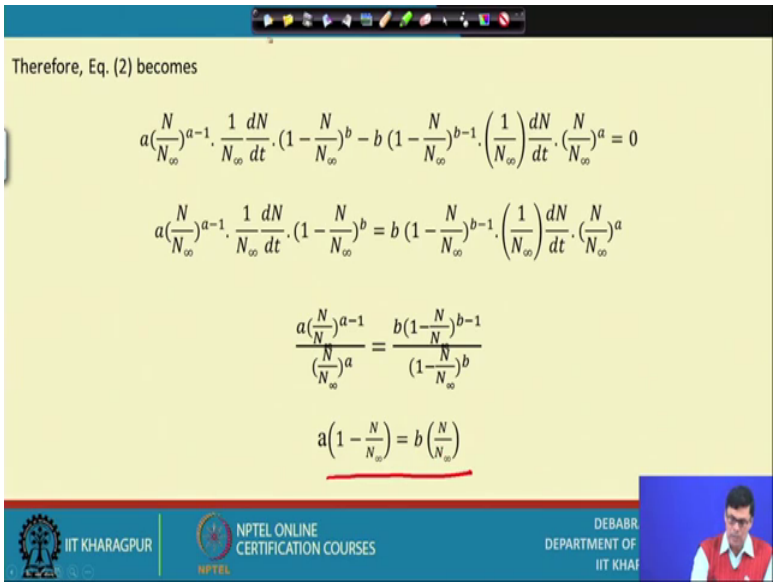

Now the; so as though now if we put these conditions that D 2 D 2 N by D t 2 equal to 0; then we can we can write this equation in this form then the equation will be becoming simplified in this form that a into 1 minus N by N infinity equal to b by N by N infinity.

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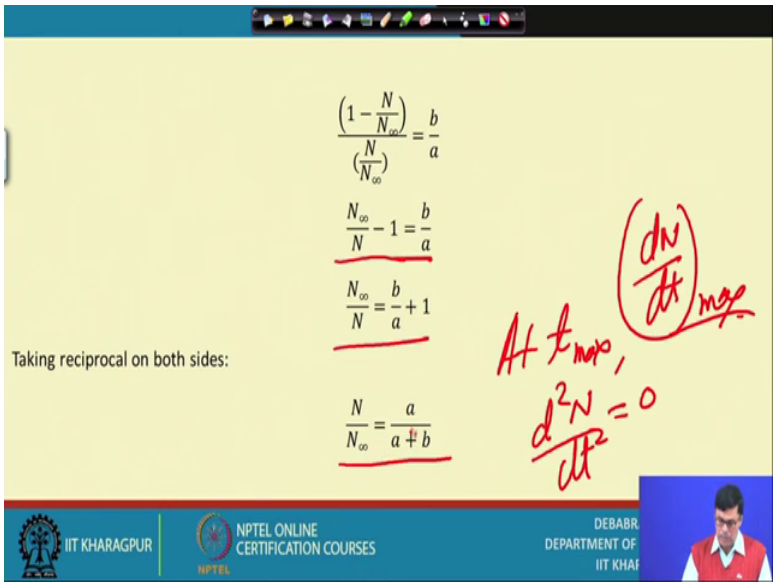

Now, when we go further, we find will come across this equation. And this equation, if we if we solve between minus 1 if you take this side then infinity I can write that a a N by N infinity equal to a plus b; that means, we can see a at t max at t max. When D 2 D 2 D by dN 2 is equal to 0 and this is occurred; when dN by dN dt is maximum this will be

this is possible than N N by N infinity equal to a by a plus b this we can we can easily find out.

Now, this thing we can put it in the previous equation that whatever we have this equation we can prove put it at this equation that the; this equation we can put it and if we put that this equation.

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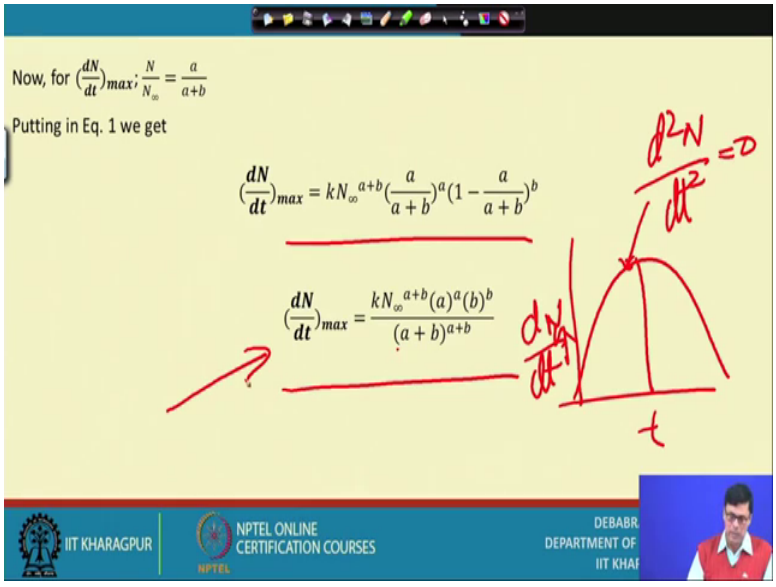

We can we can we can come across we will come across this kind of situation and then if you simplified it will come like this dN by dt maximum equal to k in alpha a plus b a to the power a b to the power b a plus b equal to.

So, it is very simple problem only the thing is that we shall have to find out that, under what condition the rate of cell mass growth will be maximum? And rate of cell mass growth will be maximum; when D 2 N by dt 2 equal to 0 you know that is because I told you I am repeating you again the dN the dt versus t when you plot.

So, here is the maximum and here at this particular point dt D 2 D d 2 by dt 2 2 N by dt 2 this should be equal to 0. If you differentiate that, we can easily find it out if you put this here you will come across this kind of if this is a very simple problem I hope if you try you can solve it by yourself.

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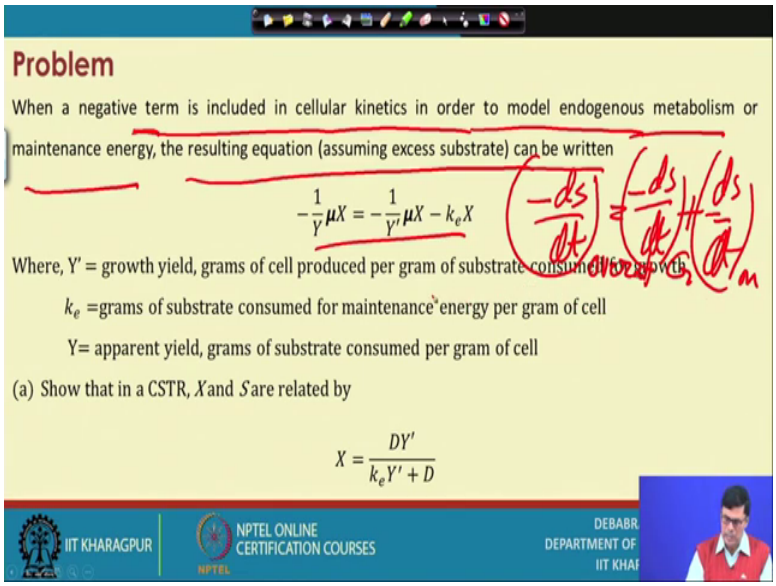

Now another very interesting problem that; I want to discuss here that we know that we come across the pirt equation what is the pirt equation? Pirt equation deals with the maintenance of the cell all what do you called endogenous metabolism of the cells.

So, what is the? I told you what is the maintenance of the cells? Maintenance of the cells means in a solution when you keep the cells in when you see the organism under the microscope you find that organisms are moving from one place to other place. And this for the movement of this organism, you required some energy and for the is some kind of protein formation or some kind of repairing of the ruptured cell we require some kind of energy. So, that use for the for the maintenance maintenance considered as the maintenance energy.

So, what I what pirt equation basically deals with that they are saying the substrate mostly goes for the cell mass formation , but a portion that is used for the grow the cell maintenance. Now, so this equation surely deals with that now when a negative term is included in the cellular kinetics in order to model the endogenous metabolism or the maintenance energy the resulting equation assuming the excess substrate can be written by this.

Now this equation we have shown you this is nothing, but D S by dt this is overall rate of substrate formation overall this will be minus dS by dt this is for growth plus da minus

dS by dt this is for maintenance of the cells. So, there are they this equation I have we have shown you that we have this equation is nothing, but this.

Now, if you divide divided by mu X then the equation will be can be written as what will be this the we can write 1 by Y.

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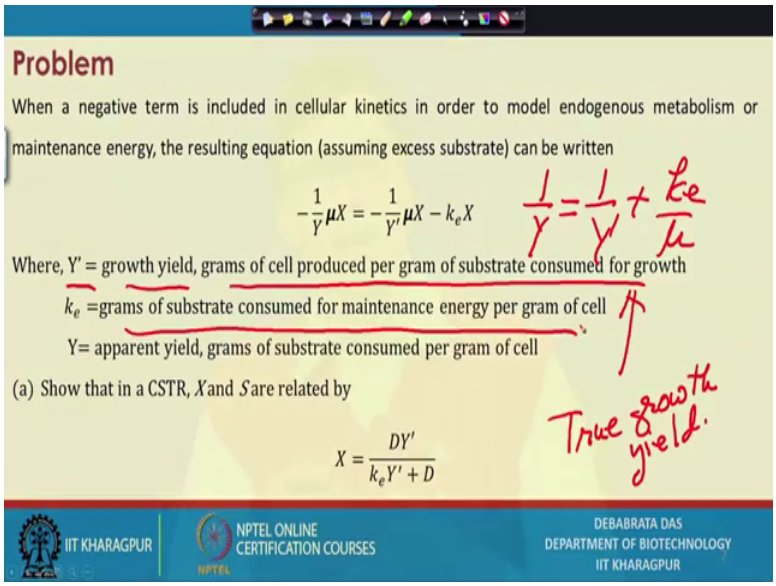

Equal to you can write 1 by Y dash plus ke by mu am I right. So, we can we can write this equation in this form. So, where Y dash is the growth yield is the krama gram of cell produced per gram of substrate consumed for growth.

Now, here we sometimes we call it true growth yield true growth yield. What do you mean by true growth yield? Suppose we already we have come across yield coefficient yield coefficient means gram of cell produce per gram of substrate consumed. Now if the if the substrate used only for the cell mass formation then we call it true growth yield.

Now, this is. So, this is the the true growth and this remain constant for the all organism and ke is the gram of substrate consumed for the maintenance energy per gram of cell. Now here rate of substrate consumption for the maintenance of the cells is; what is this all always proportional to the concentration of the cell because more cell the mode will be maintenance energy requirement less cell less will be the maintenance energy.

So, what we can write that we can write that minus dS by.

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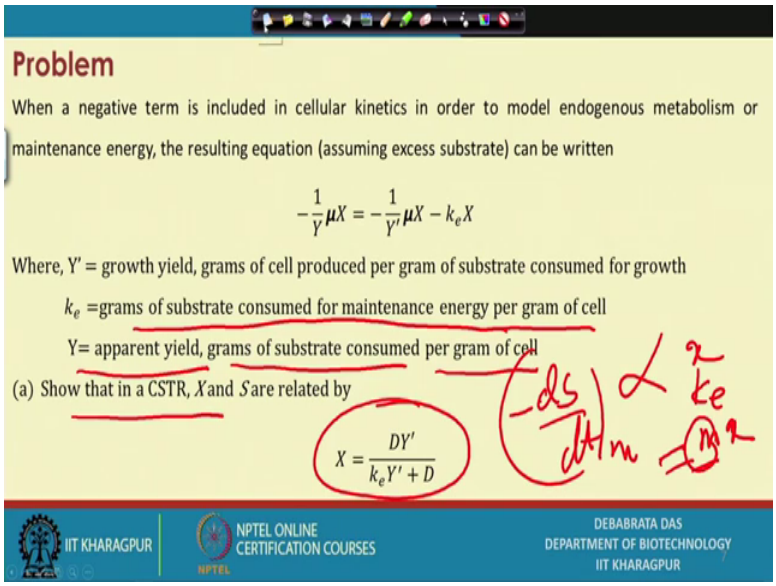

Dt this is for maintenance this should be proportional with the cell mass concentration. So, this should be equal to m into X; what is m? Maintenance coefficient now here this has been substituted by ke now k is the gram of substrate consumed for maintenance energy per gram of cells.

Now, Y is the apparent yield sometimes we call it overall yield coefficient and gram of substrate consumed per gram of cell mass formation. So, so what we shall have to do we shall have to show that in a CSTR or chemostat X and S are related by this we shall have to prove that this is related I will by this.

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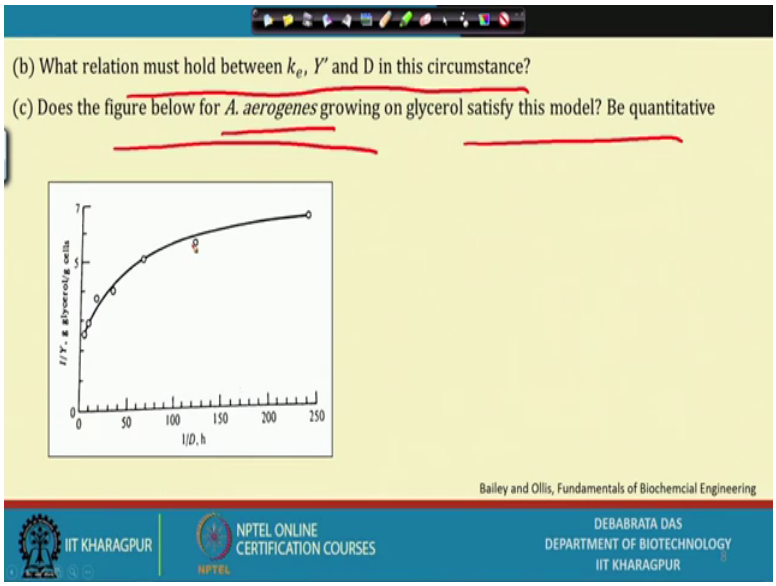

And then what is the relation? What is the relation between ke Y dash? And D in this circumstances and does the figure of below the as aspergillus aerogenes we growing in the glycerol satisfy this model.

So, they have the you know that that aerobactor aerogenes this is kind of organism that is that when you plot 1 by Y versus 1 by d; D is the nothing, but the dilution rate they get this kind of correlation and we shall have to prove we shall have to find out whether it satisfied this pirt equation pirt models. So, this is this we shall have to prove.

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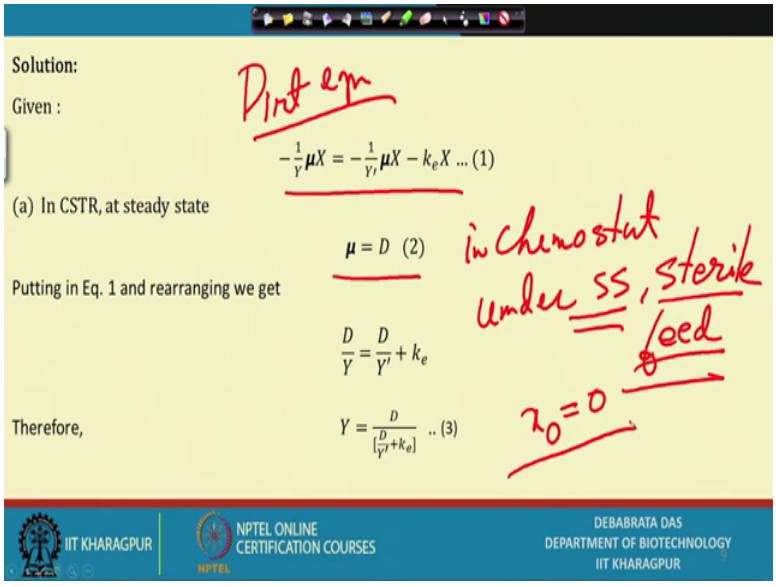

Let us see how we can solve that? Now, this is the basic equation is given that you know that this is the pirt equation we know this is the pirt equation this is a given in the problem; and we know mu equal to D in a in a chemostat under steady state condition under steady state condition and the sterile feed.

What is the steady state condition? Let me repeat it again that with steady state condition means when the concentration of the different material present in the reactor remain unaltered remain constant steady with respect to concentration. When it is changed this is un unsteady state as for example, suppose you are putting the substrate in the reactor and if the substrate concentration keep on changing; what do you call it unsteady state? Now if the substrate concentration remain constant does not change with respect to time then we call it steady state condition same thing applicable to the cell mass same thing applicable to the product formation.

So, that is the steady state. So, under steady state condition sterile feed; what does it mean? When the media content does not have any kind of cell mass X 0 equal to 0 then we call it sterile feed.

Now, here this equation I can write like this that we can we can divide divided by X then we have this if you divide by X then we will be having the equation what mu.

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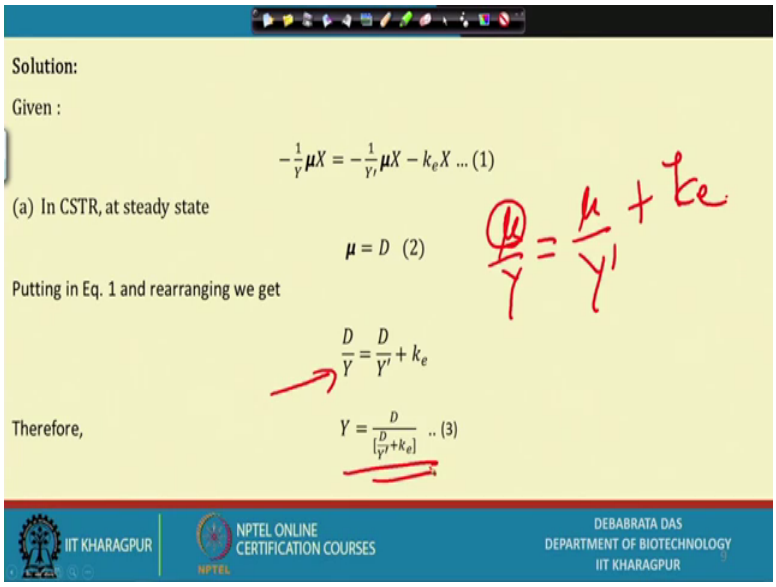

By Y equal to mu by Y dash plus k am I right and then D equal to mu. So, if you replace mu by D though we will get this equation and finally, we will come across this equation.

So, what the problem that we have this kind of equation.

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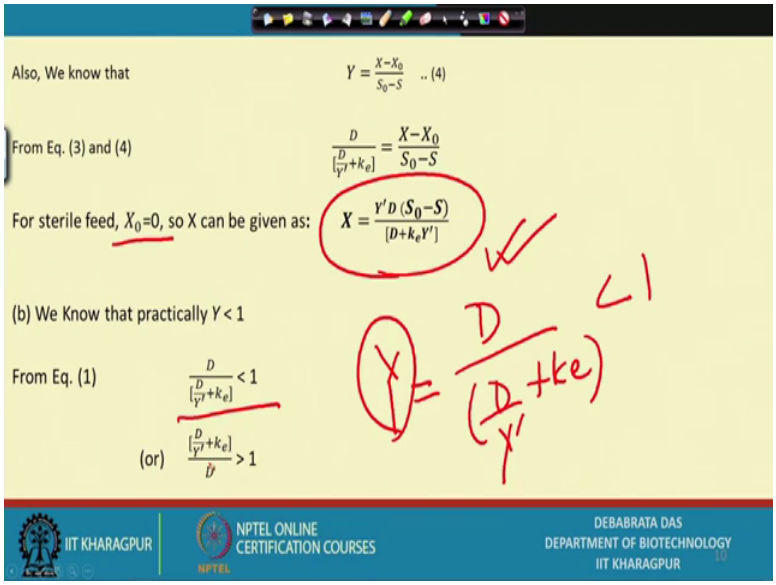

Then what do you know Y Y is the overall yield coefficient average yield coefficient this is equal to X minus X zero by S zero minus S now then what we can write D by D Y dash by ke equal to this yield coefficient.

So, now if we put sterile feed X zero equal to zero then X can be written as this is multiplied by S zero. So, this is what a portion of the problem is looking that they given that you have to prove that X equal to Y dash a and D S zero minus this divided by D plus ke plus y. So, this can be easily proved.

Now, question comes what is the relationship between D ke key and k dash. So, what we can write that that what we have go with here we what we have find out Y equal to Y is the yield coefficient am I right this is equal to D by D Y dash plus ke now why yield coefficient always less than one.

So, we can we can always write this is less than one am I right if this is exactly what is the written there then if we if we do the reverse then if you bring it at the top then what will happen this will be greater.

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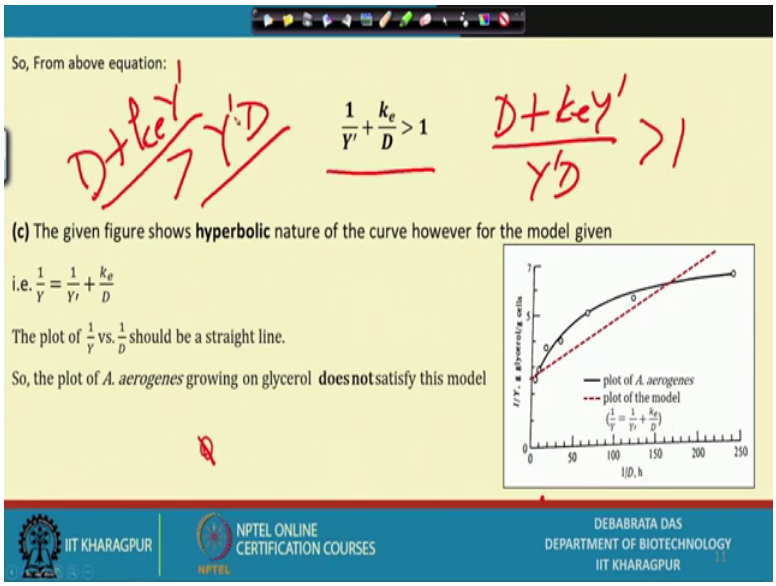

Than one and then we can have this relationship as they have this relationship that we have this is that 1 by e. Now this we can we can we can do this further simplified that what you can write this is D plus ke into Y dash by Y dash into D am I right this is like this I can write like this.

now I can I can I can I if I if I multiply both side Y dash by D then it can be written as D plus ke Y dash you should be greater than Y dash D am I right then if you then the the then if you if you divided by Y dash then what kind of correlation you will you will get Y dash if you if you do that then D. Now then the same kind of relationship so, this kind of correlation ship that hold goods we can write that this kind of correlation ship between the D k and Y dash. We can have this kind of correlation or you can divide by Y dash then you can you can bring this one term this side and make the things little bit simpler.

. So, this is how we can find out the correlation between Y dash k e and D and last part of the problem that we have that you know the in case of a areobactor aerogenous they found the correlation between Y 1 by Y by 1 by D because this is the equation. We have because if you look at we have we had this equation am I right this is the equation we had.

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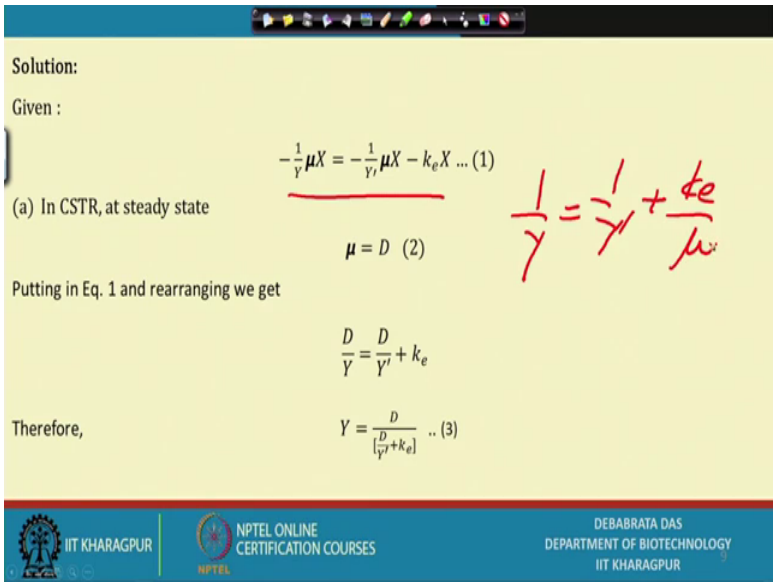

Now, if you divide by mu X then what will get Y 1 by Y equal to 1 by Y dash plus ke into mu am I right the this is exactly what we wanted to do it here though we have only this we know under steady state condition and sterile.

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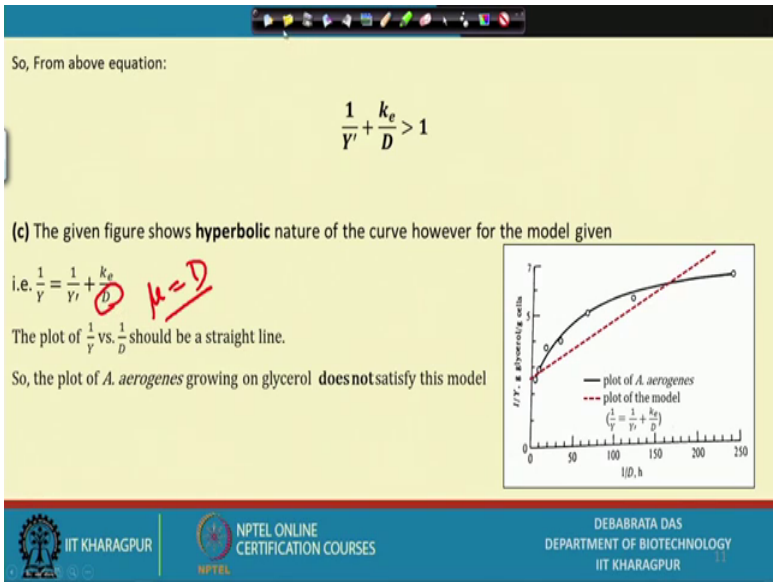

Feed the mu equal to D. So, if you put mu equal to D. So, we can write here D this is the only the difference.

Now, here what are the variables? Y is the variable. So, yield coefficient and D is the variables and ke is the maintenance coefficient and the Y dash is the true growth yield

that that are the constant am I right. Now if we if we plot 1 by Y versus 1 by D, then they found this is kind of correlation they obtained in the aerobactor aerogenous.

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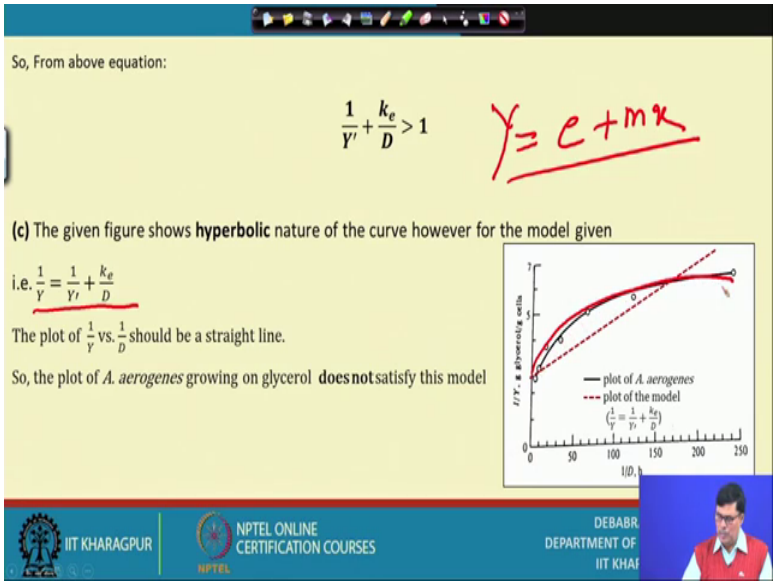

But as you know that if you look at this equation what is this equation the equation equal to Y equal to c plus mx and this is a basically a straight line equation. So, it should be a straight line instead of straight line this is a kind of hyperbolic type of equation hyperbolic kind of relationship that we have. So, what I want to comment here that this relationship does not satisfy this model, because this what is the curve that is showing here? That that is not satisfied this model that should be the answer.

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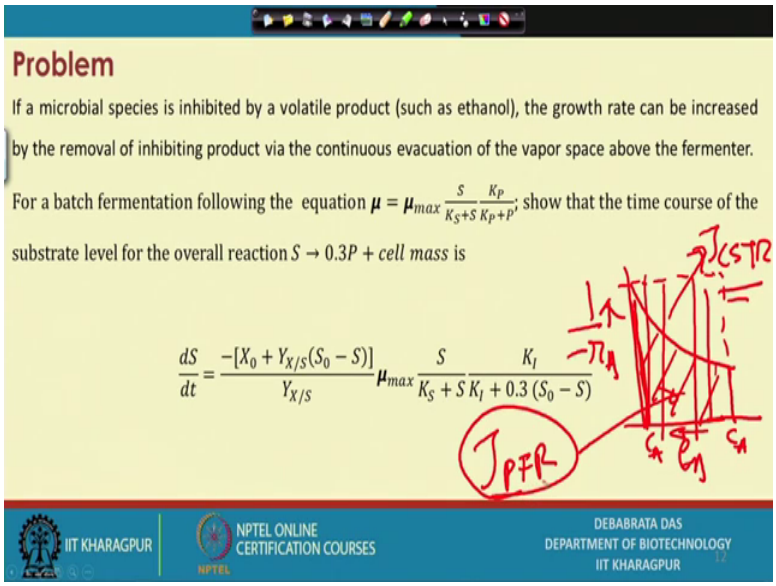

Now let us go to the that another problem that is also very interesting that that a microbial because in case of microbial fermentation process we have come across different type of inhibition. We come across the substrate inhibition we have come across the product inhibition; I when I told you can remember in case of substrate inhibition. Usually we preferred the fed batch reactor.

But in case of product inhibition we preferred the where the plug flow reactor because of why because we have seen that that when we plot 1 minus ra versus ca this kind of plot we have ; that means, when substrate is degree this is ca 0 am I right and this is your ca this is this might be your ca.

So, when is substrate concentration decreases that 1 by ra increases; that means, ra decreases now as the substrate concentration decreases why the rate of reaction decrease because mainly due to the product inhibition.

And we have seen that in case of this if you have this kind of correlation we find the area under this curve this is; what is the area under this curve? Is tau plug flow reactor am I right. So, this is much less as compared to this is this the what is this is the this is the a the area under this is a the rectangle what do you call tau CSTR. So, tau CSTR will much higher as compared to tau plug flow reactor. So, we will we will preferred plug flow reactor am I right. So, in case of product inhibition we usually recommend plug flow reactor.

Now, this particular problem deals with some kind of product inhibition. And we shall have to develop some kind of equation out of that let us see how we can do that in a microbial species is inhibited by the volatile products such as the ethanol we know that a high ethanol concentration that you know growth rate of the organism that will be inhibited.

Now, growth rate can be increased by the removal of the inhibiting the product via continuous evacuation of the baker vapor space above the fermenter. So, what we can do that you know that if we applied vacuum we know the boiling point of ethanol is less as compared to that of water am I right.

So, if we if we if we applied vacuum that even at the normal temperature that a alcohol will be vaporized this out now if alcohol is vaporizes out then that product inhibition due to certain concentration of ethanol can be overcome that is exactly they have mentioned here.

Now, for a batch fermentation process following equation the is there that mu equal to mu max S by Ks plus S Kp plus Kp plus P and show that the time course of the substrate label the overall is this the S equal to point 3 P plus cell mass and if if S is like this this kind of correlation if this holds good.

Then that then the equation will be this his equation that equation will be this equation this is the dS by dt equal to X 0 plus Yx by S S 0 minus S Y X by S mu max and the S by Ks and this the equation and ki is the kind of inhibition constant that we shall have to prove this is the equation this is the problem that we have now let us see how we can solve it.

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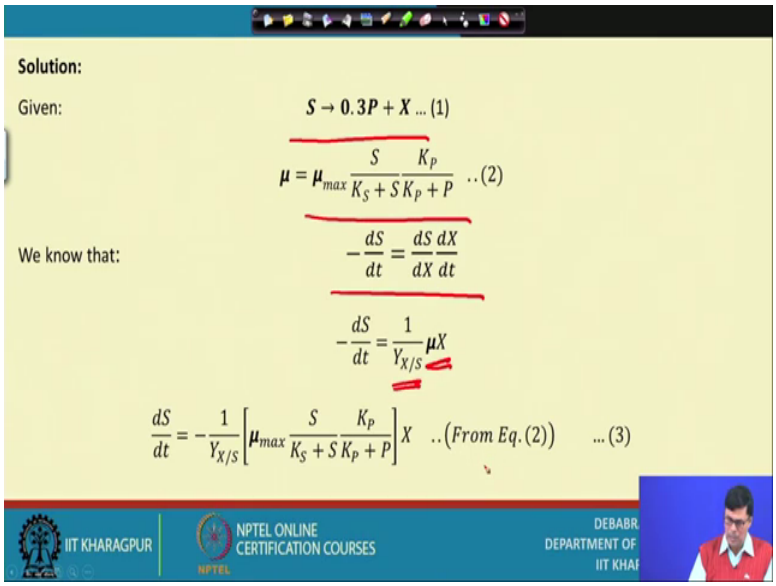

Now, this is the stoichiometry is given and this is this the stoichiometry is like this S equal to substrate equal to the 0 P plus X the X is the cell mass and this is the equation that is given and we know dS by dt; how we can write dS by dX and dX by dt am I right and dX by dS is equal to Yx by S that we can all it this is called yield coefficient and dX by dt we can write mu into X is the rate of growth of the cells.

Now here that. So, dS dS by dt what we can write that 1 by 1 by this and this thing I can substitute here that in place of mu I can substitute into X this we can get this the two equation we can substitute you can get this equation.

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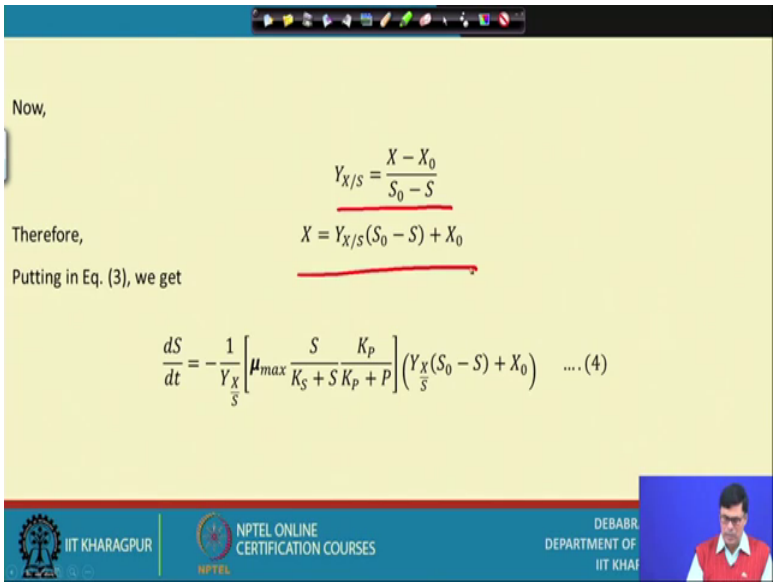

Now, Y x by S is X X minus X 0 by Y S 0 minus S am I right the X equal to what X equal to Yx by S S 0 minus X. Now if we put this in the previous equation. Then this equation if we put it then what we will what we will get we will come across this equation dS by dt equal to one by Y X by S mu X S by Ks plus S Kp plus and this X will be substituted by this I can write like this now this will cancel and this.

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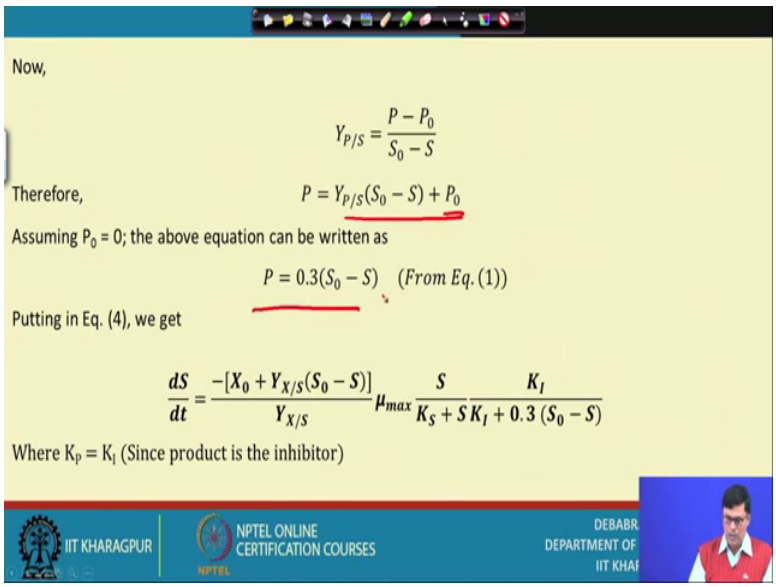

And Yp by S how we can write this is a gram of product form per gram of substrate consumed P minus P 0 by S s 0 minus S. So, P equal to I can write Y P by S is 0 minus S

into plus P 0 am I right and P equal to the stoichiometry is given if P equal to 0 then we can we can we can write P equal to 0 we can write then P equal to this point three S 0 minus S 0 minus S that we can write.

The equation if we if we look at the equation before you that what we have done before that you know that this equation. So, we have we have we have this S equal to 0.3 into X. Now this equation we can we can put it here. So, and this equation we can write in this form then we can put this value in this equation we will finally, we will come across this kind of equation. So, we will come across this kind of equation I hope this is how we can solved this problem.

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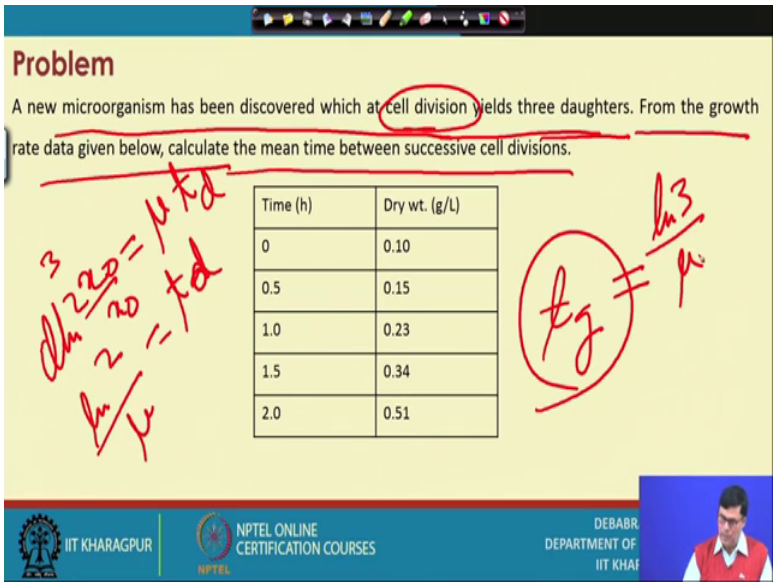

Now, another very interesting problem is that; we have come across the generation time am I right. And we also we come across the doubling time of the cell. Now when you talk about the doubling time; what is doubling time? The time required to double the cell population. What is generation time? The time required for the one cell division.

Now, here if we look at this problem that a new microorganism has been discovered which at it is cell division. Cell division means this is the generation time it gives the 3 dotted cells and then from the growth data below calculate the mean time between the successive cell division ; that means, we shall have to find out the generation time tg who is we will have to find out.

Now, in the doubling time we have seen; what is the equation we have seen dln X by X 0 equal to mu into t am I right. Now in case of generate that doubling time this will be 2 X 0; then the equation will be ln 2 by mu this will be equal to td am I right.

Now, here in case of in case of generation time what will this is the this is called this is a one cell produces three cells in one division. So, equation will be little bit different the ln three by mu am I right.

Now question comes how we can determine the value of mu.

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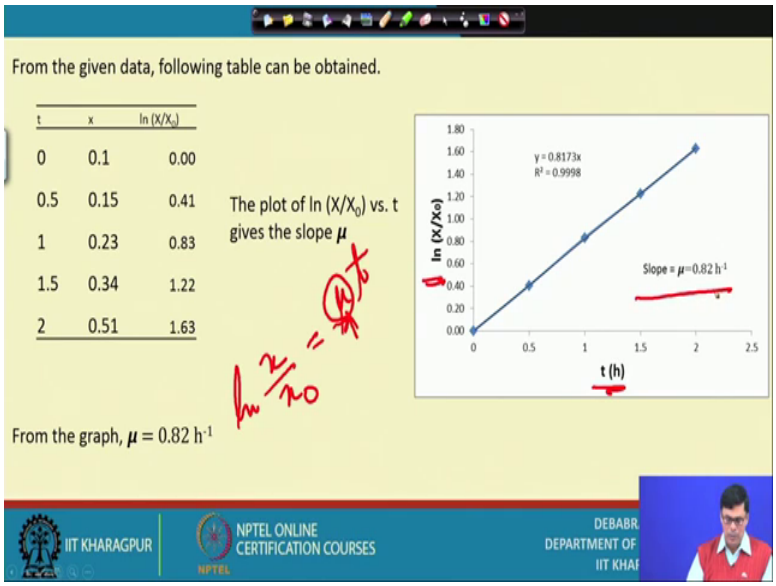

Now, we have we have here in this problem; what is at the different time? This is the batch system we can easily estimate the cell mass concentration this is the cell mass concentration we have. Now from that we can now; what is the equation that we have? That ln X by X 0 equal to mu into D am I right.

Now,. So, here if we assume this is mu is constant because in the log phase. So, we can we can write that X by X 0 we can we can write this value and this value you can plot in the ordinate and t in the abscissa then we will get a straight line and slope ; obviously, slope will give you the value of mu slope will this is exactly what we can find out the value of mu. So, you can easily find out the value of mu.

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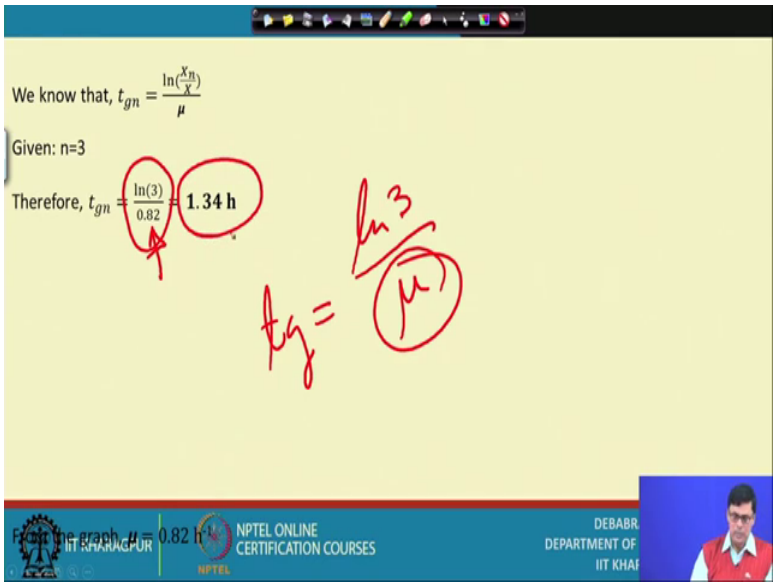

And then as I as I mentioned that in pirt generation time. So, what is that equation this is like this ln tgi told you tg equal to ln 3 by mu. So, we have already estimated the value of mu we put this value of mu and 3 ln 3 is there then we can find out the generation time. So, this is a very simple way we can we can easily determine the generation time of the cells.

So, in conclusion what I want to tell that in this particular lecture we try to understand; how to find out the time at which the maximum rate of growth of the cells take place and we have given you some kind of logistic equation the some kind of equation proposed by Konak and from that equation we find out that t max value and the how the maximum rate of cell mass formation can be expressed that we try to discuss then we discuss some kind of product inhibition problem and we try to develop some kind of equation and lastly we try to discuss that you know that kind of generation time of the cells.

Thank you very much.