Aspects of Biochemical Engineering
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Lecture - 41
Kinetics of substrate utilization, product formation and biomass production of microbial cell-XI

Welcome back to my course Aspects of Biochemical Engineering. And last couple of lectures we try to discuss the different numerical problems on the kinetics of substrate utilization, product formation and biomass production using microbial cells. Now today this is this is the last part of the numerical problem; that we are going to discuss today and in last couple of classes we try to tell you that; how to analyze the batch system? How to analyze the chemostat? How to analyze the plug flow reactor? And then also we talk about some inhibition problem substrate inhibition, product inhibition of this we try to discuss.

And also we try to find out the procedure or you know that techniques through which we can find out the kinetic constants like maximum specific growth rate saturation constant true growth yield at the maintenance coefficient. Now today this lecture we try to cover little bit different, but you know what we want to tell here; that you know the how the we already discuss;.

how the best data can help you to find out the data there for the chemostat process as for example, from the a from the batch analysis we can find out that at a particular dilution rate what would be the cell mass concentration in a chemostat. We can easily find out from the batch process that that then we try to discuss the different type of systems as for example, that if we use the multiple reactor in series; how you can find out the cell mass concentration, because we have seen before that in case of cascade type of fermenter that you can be easily determine the substrate concentration in different reactor cell mass concentration in different reactor by doing the substrate balance cell mass balance.

But for when you do that then we have to come back for the different type of quadratic equations then you shall have to solve it, but you know this problem when you the problem that I shall discuss; I shall show you how graphically you can find the cell mass concentration obtain n number of reactors and also what should be the pattern that we

have in case of diauxic growth of the cells and this is the diauxic growth we know it is special type of growth because in the if the media contains more than one carbon source some cases until unless one carbon source is totally exhausted other carbon source will not be utilize.

So,. So, after this again we shall go for the chemostat process to find out the where the cell that what is that time required for a particular amount of substrate conversion and all this thing by using Zymomonas mobilis for a particular product formation we try to form found out and in after that at the end what I am planning to discuss that how this you taking piret model.

We can use just to find out that when at what dilution rate you can have we can have the maximum rate of product formation and also on the from the D max value we know d max equal to mu max into 1 minus root over ks by ks by s 0 how we can find out the maximum the dilution rate at will be we will get the maximum rate of cell mass concentration.

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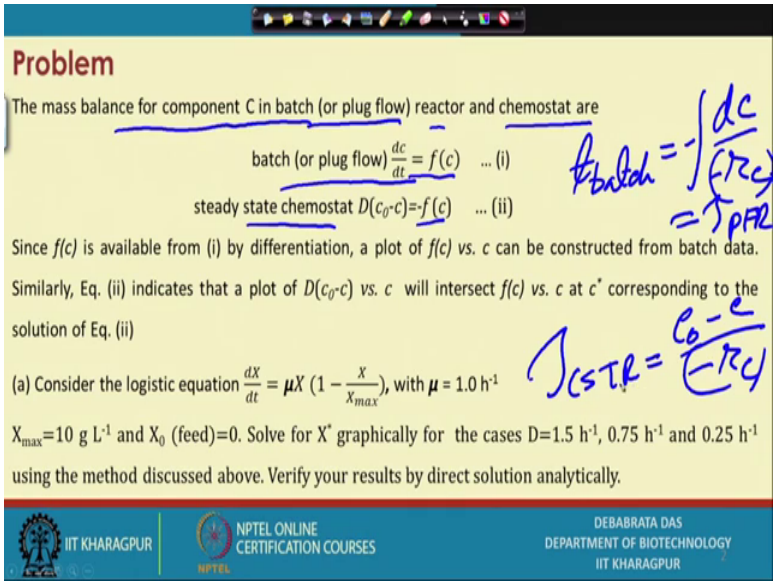

So, when is let us start with this first two problem that we have and if you look at this problem the that here that; the problem says the mass balance of how for component c in batch or plug flow reactor and chemostat are this they for the batch and plug flow reactor dc by dt equal to fc that is the function of c. And in case of steady state chemostst d c 0 minus c equal to minus fc because we have we have seen in case of chemostat process.

In case of batch or batch process t batch in case of t batch; what we can write t batch equal to? What we have seen that minus dc by minus rc am I right this is the this same as your tau plug flow reactor plug flow this we have seen, but the in case of tau cstr. We have observed this is equal to c 0 minus c by minus rc the rate of degradation of c.

Now, this is the exactly that they try to find out that this is dc d into c 0 minus c function of c and this is like this. Now since the fc is available from the by differentiation a plot of fc and versus c can be constructed for the batch data and equation two indicates the plot of c 0 minus c versus c will intersect fc versus c as c star correspond to the solution c. Now, what does it mean? Actually let me let me explain, this show in a batch process there is suppose when you talk about the batch process.

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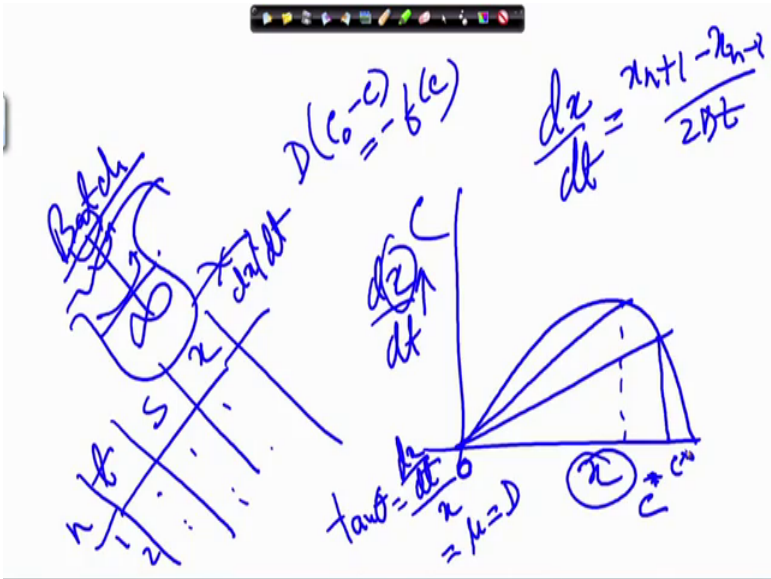

Now, in the batch process, how you operate with this is the reacted that we have and this is rho rotating like this though time to time; he draw the sample. So, what you can do you we can at different time we can have different substrate concentration and different cell mass constant am I right. And then we can find out dx by dt and I told you dx by dt; how we can find out dx by dt equal to x n plus 1 minus x n minus 1 divided by 2 del del t; that is the n is the number of sampling 0.1234 like this.

Now, in this in this particular batch data; what we can do? We can plot dx by dt versus x am I right; and then we are have the plot like this. Now what this problem says in the

CSTR? What you have dc 0 minus c equal to minus function of c am I right. Now; what is the what is the slope slope stands for?

This is if you consider this is 0; then this slope with the tan theta is tan theta is equal to what you have this and this altitude versus base that is dx by dt divided by x am I right and this is equal to mu and we know; in case of in case of chemostat under steady state condition and sterile fed mu equal to d.

So, this; that means, this is the particular dilution rate. So, we can have different dilution rate we can we can have different. So, this they in suit of x they are considering c this is replaced by c. So, this is this is indicating the c star indicating the c star. So, this is the; this is the how in this problem? Now, we can we can make the correlate the batch process with the chemostat process.

Now, the problem is that; considering the logistic equation dx equal to by dt equal to mu X 1 minus x by X max mu equal to 1 hour inverse x max; X max is the maximum cell concentration 10 gram per liter and X 0 see it is 0 solve the x star graphically for the case when d equal to 1.5 hour inverse 0.75 hour inverse 0.25 hour inverse using the method discuss above and verify your results directly from the direct solution analytically.

So, what we shall have to do we shall have to solve it both graphically as well as the analytically though let us see how we can solve it.

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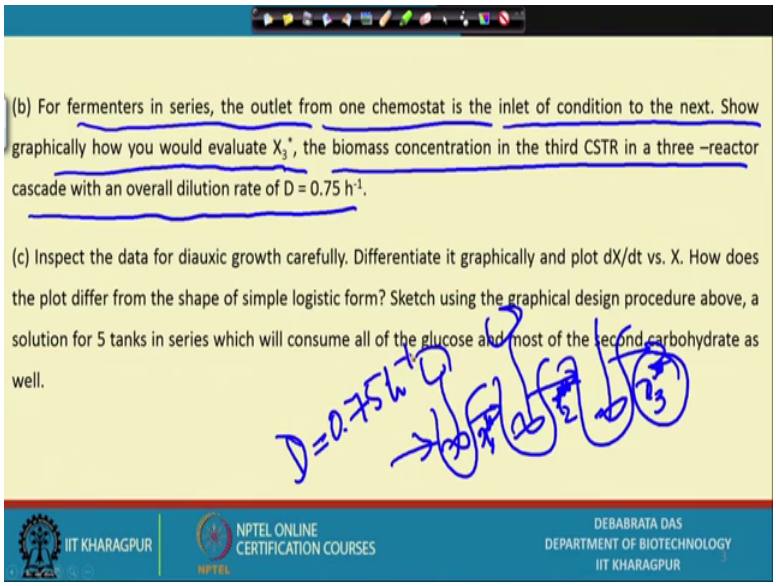

And you know other portion of the problem is that for the fermenters in series the outlet from one chemostat in the inlet condition for the next and. So, graphically; how would you evaluate X 3 dash? The biomass concentration of the third CSTR in three reactor cascade with a overall dilution rate of 0.75.

So, we shall have to find out that; suppose we have this is 1 CSTR and this is the another CSTR and this is the CSTR that we have their connecting to each other. So, here we have X 1 star here a at the steady state condition X 2 star and here you have X 3 star. So, we shall have to find out; what is the X 3 star? When D equal to 0.75 hour inverse this is the problem that we have.

Now, last problem that inspect the data on diauxic growth carefully; hence differentiate is graphically and plot dx by dt versus x, how does this plot differ from the same of simple logistic form sketch using the graphical design procedure above a solution of 5 tanks in series which consume all of the glucose and most of the second carbohydrate as well.

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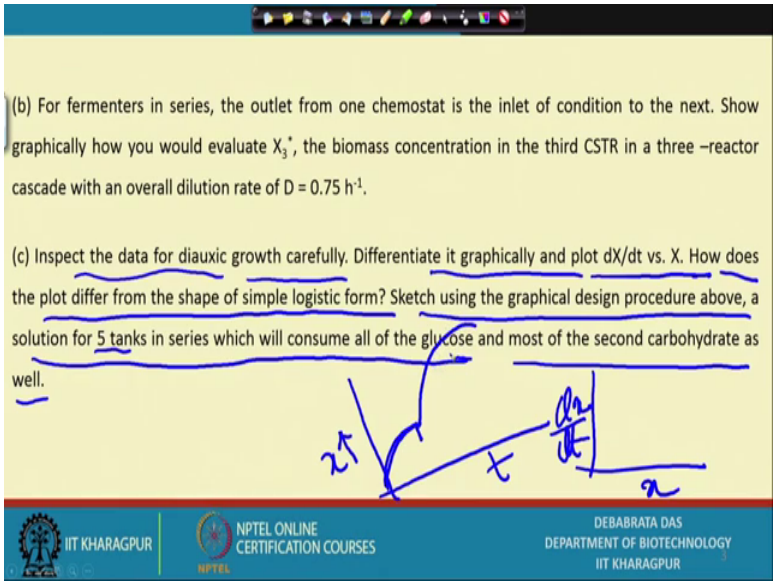

So we know the diauxic growth means what diauxic growth means if you have the cell mass concentration with respect to time. So, it is going like this and then until unless first carbon source is totally exhausted, the second carbon source need not be utilize the nature of diauxic growth is like this; the during this diauxic growth what will be the nature of the graph if we plot dx by dt versus x? What should be the nature of plot that we shall have to find out?

And then we shall have to find out graphically that you show me under what circumstances if we use 5 tanks in series it consume not only the first carbon source, but also it will consume the second carbon source.

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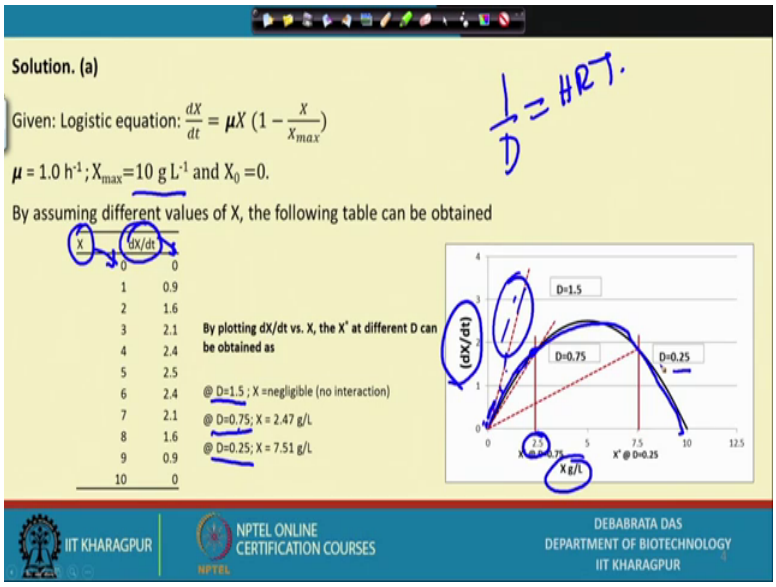

Now this is the this the solution that we had the logistic equation that has been given dx equal to mu X a 1 minus X by X max.

Now, what we can do here every all values are given? The mu value is point 1; 1 hour inverse X max is 10 gram per liter and in series. So, I we shall have to; what we shall? We shall have to do we shall have to plot dx by dt we shall have to plot dx by dt versus x am I right. So, what we can do? We can may prepare a table in the table. We have 2 column 1 is a x another is dx by dt.

So, this dx by dt will come here x will be here. Now we know maximum value of X max you see if the 10 gram per liter. So, we can assume this 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. Ee can assure this respected value of dx by dt; we can find it out and if we have this then we having this kind of plot you know this plot we will be having we can we can have a plot like this. And once we have this plot; then we shall have to find out that that different dilution rate 1 is the D equal to 1.5 hour inverse 0.75 or a 0.25 hour inverse? What will be the x value?

Now, if you if you if you considered the D value is 1.5. We find the tan theta is a little bit high. So, it will not touch the curve. So, we can we can we can assume that this is the situation where and the cells will be was out from the system; because you know this we can easily visualize from this that you know your that you know that, this is this from this one particular figure that you know. This is the there should may be no cell present in the reactor that; when we do the; it analytically then also we can justify, how it happens?

But, when we have 0.75 then it touches the curve here. So, your cell mass concentration would be 0.2, 0.5. Now when you have 0.25 dilution rate, then is a 7,5 now we know 1 by d equal to hrt am I right. Now; that means, if the dilution rate is high that then you are allowing the cell to resize in the reactor for smaller period of time; and if the d value is low ; that means, you are keeping the cell for longer period of time, then naturally you have more cell growth in the in the reactor.

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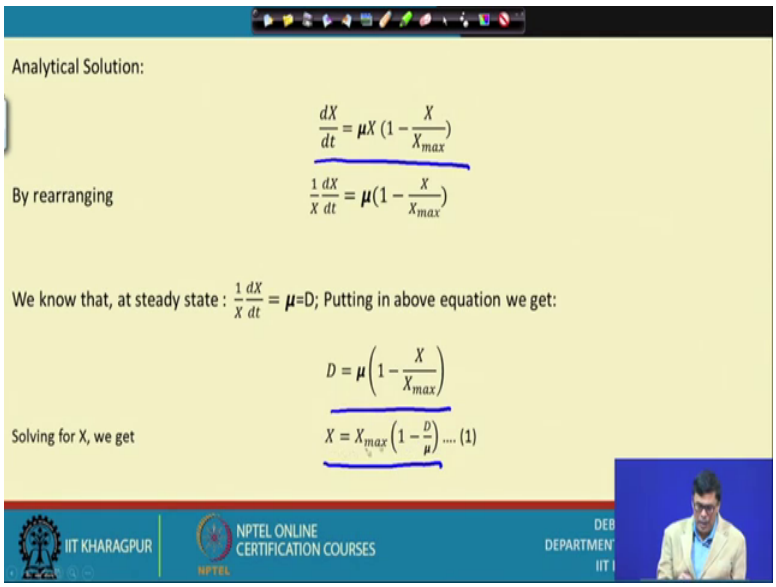

Now, next problem that we have that you know that we have this analyticals analytically a we can solve like this that this is the logistic equation that we have. Now we can bring this side, then may we can write mu equal to D, then will have the correlation like this and then we can find out the expression for X equal to X max equal to we can write in other way.

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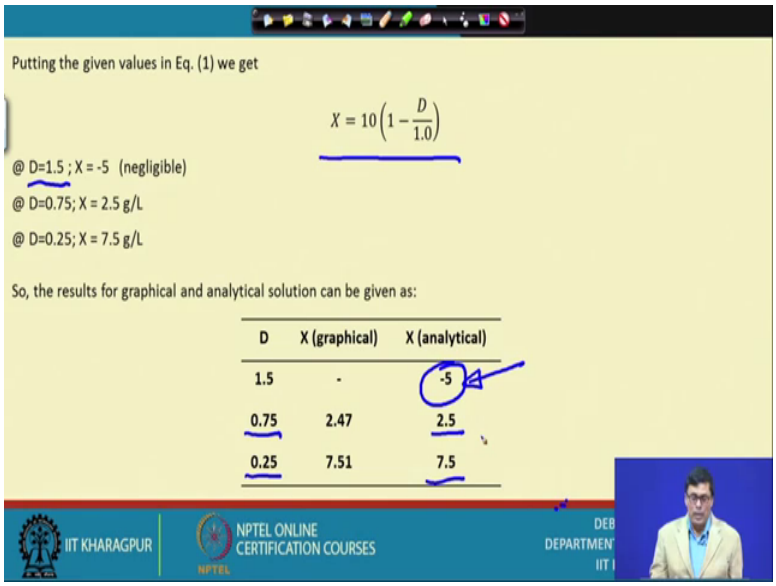

We can write in this form, then we have this equation we have we have X equal to 10 X max is the 10 value 1 minus d by 1 1.

Now, in this equation; if we put the value of D, 1.5 directly in this equation is value is X value is coming about minus 5 which is which is not possible am I right. So, this is; that means, this is kind of a situation when that is any cell present in the reactor , but 0.75 it is 2.5 and 0.25 it is 0.75.

So, and the graphically also we find almost the same result. So, there is there is imbalance between the between the graphical and the analytical that solution of this particular problem.

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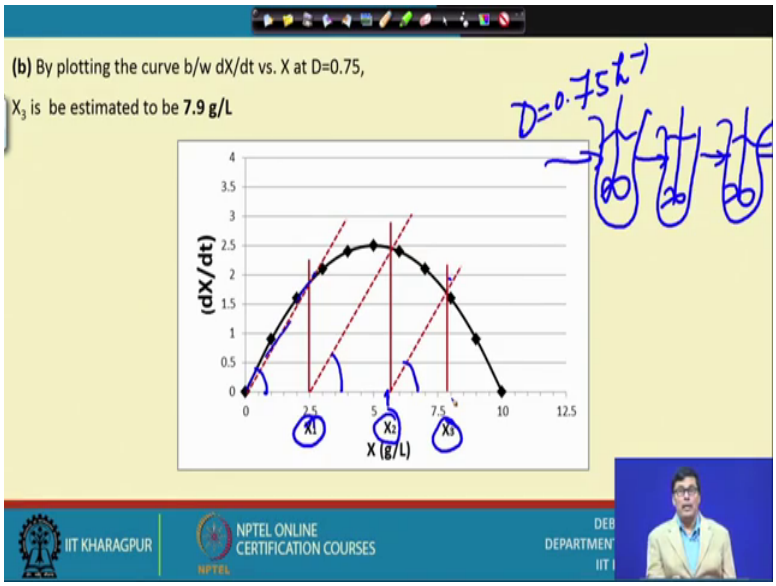

Now, if you if you if you look at the second case that what is saying that same curve we have; now 0.75. So, I as I told you there the title use the cascade fermenter that mean three CSTR that will be in series this they are they are connecting to each other am I right and they have the same dilution rate D equal to 0.75 hour inverse.

Now, here if you look at this angle is 0.75; this is 0.75 and this is 0.75. So, this same s angle that we mentioned that is 0.75 dilution rate here. We have the value of X 1 and then here we have the value of X 2 this is the point that we have and here we have the value of X 3. So, graphically we can easily find out that after third reactor in series what will be the concentration of the reactor and the concentration of the cell mass under steady state condition is very easily find out.

Now, if you go to the last part of this problem what is saying that we are?

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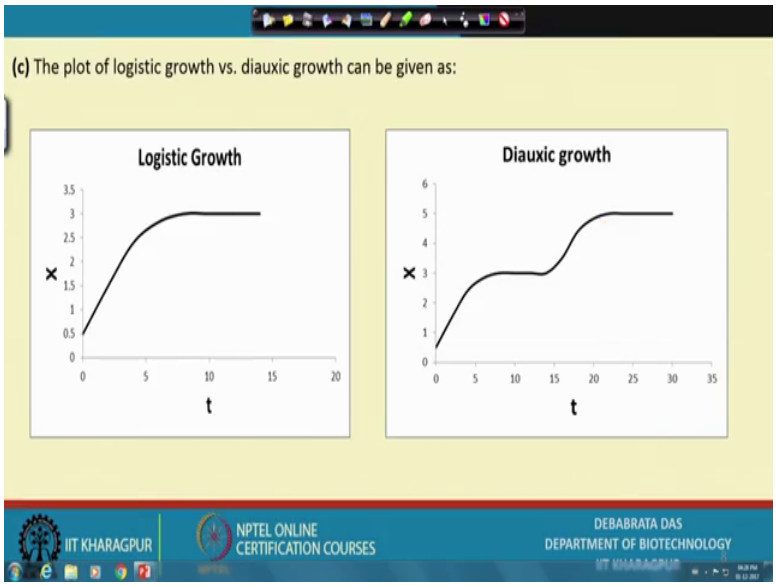

We are talking about diauxic growth of the organism, they usually the logistic growth is like this that you know that expositional grow like this with respect to time, but in the in the diauxic growth I told you that if the media comprises of more than 1 carbon source. Some of the organism has the characteristics; the until and unless the first carbon source is fully utilize. The other carbon source cannot be utilize due to the; the nature of metabolism of the particular organism.

So, this is the nature of diauxic growth now if we if we write.

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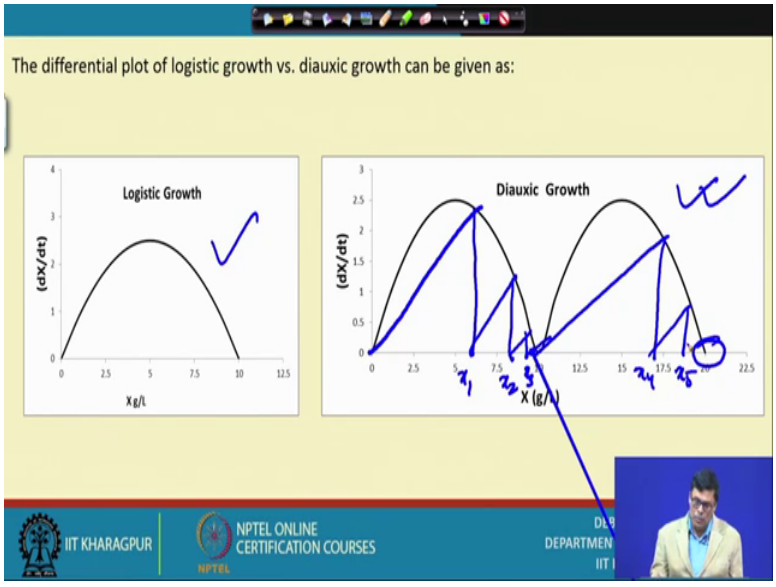

This this diauxic growth in the in case of this dx by dt versus x we will get the nature of the plot is like this. So, this will be the nature of the plot logistic equation. We have and the diauxic growth we have this kind of plot that we have now; what we can do here? Now they are saying that that we shall have to done 5 reactor in series.

So, in series. So, and we shall have to prove under what circumstances all the carbon source will be exhausted; first as well as second. Now let us take the example, suppose we have we maintain this dilution rate then we have we can have this we can have this we can have this, then we can we can have this we can have this. So, if we look at this is X 1 this is the X 2 this is the X 3 this is might be X 4 where they X 5 am I right.

So, we assume the most of the substrate consumed here am I right. So, what we shall have to do we shall have to then we shall have to change the curve little bit more because you know the D value will decrease with the hydraulic retention time. We shall have to increase little bit. So, that if you do like this. So, we can we can see. So, you will find that X 5 when most of the carbon source will be exhausted.

So, this is the this is the trajectory; how we can find out then under what circumstances all the carbon source will be exhausted. So, we can easily find out if you do the analysis of the particular process.

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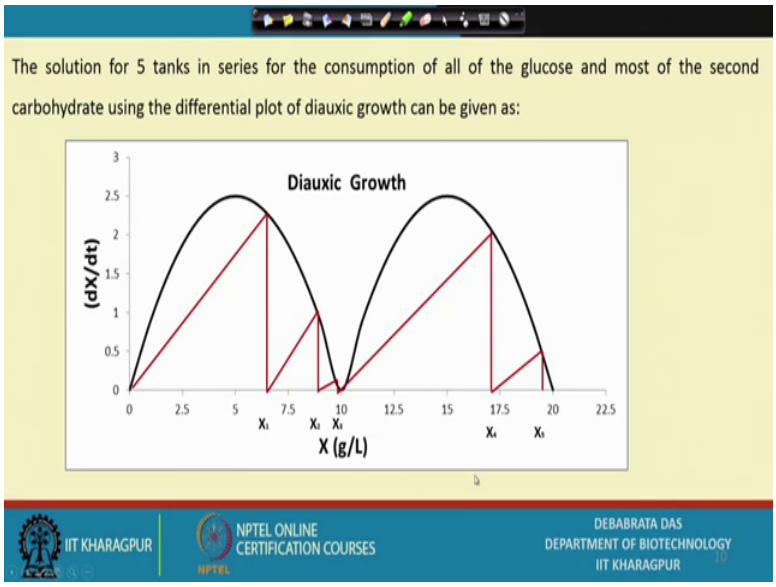

Now, this is the this is where here we have the solution I hope you understand that this is the this is like this 1, 2 this is X 1 this is X 2, X 3, X 4 and X 5. So, when it comes here we can assume that most of the carbons whose will be exhausted this is how we can solve this problem.

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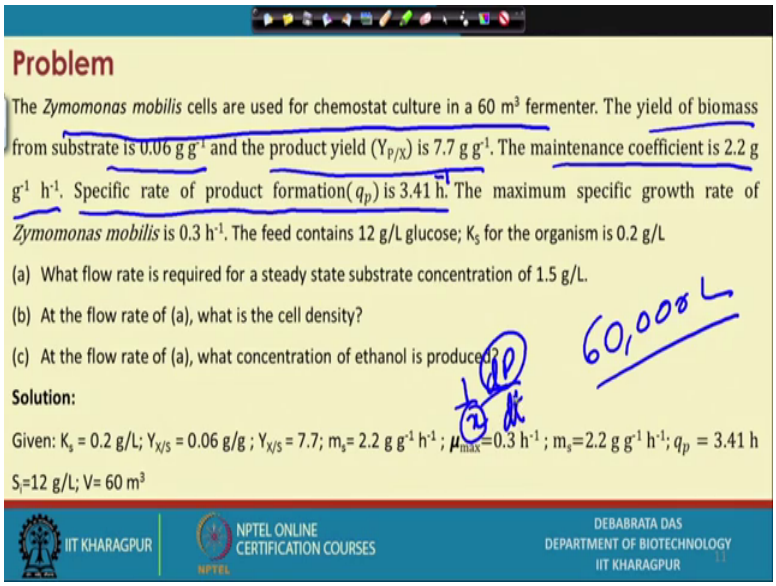

Now, let me go to the second; second problem which is the also very interesting we know Zymomonos mobilis that we use for the alcohol fermentation process. Now in Zymomonos mobilis a cells are use in a chemostat a 60 cubic meter fermenter 60 cubic meter means 60,000 liters am I right.

Now, yield of biomass is about 0.06 gram per liter. So, we know that organism under anaerobic condition they produce the alcohol am I right and under anaerobic conditions. The yield cell is very less as compared to aerobic process aerobic process cell yield always very high. So, that is why you see this is 0.06 gram of cell per gram of substrate consume; the product yield is 7.7 gram per gram the maintenance coefficient 2.2 gram per liter hour.

Then specific rate of product formation is 3 3.41 hour and this should be our inversion on you can make little bit correction this specific rate of product formation unit should be hour inverse and becomes specific rate of product formation is what dx by DP by dt am I right. So, this is also gram per liter this is also gram per liter.

So, the, what will level this will be time inverse the maximum and the maximum specific rate of growth of Zymomonas mobilis is 0.3 hour inverse. The contains 12 grams per liter of glucose and ks of the organism saturation constants 0.2 gram per liter the; what is the flow rate is required to the steady state condition? A concentration of the substrate will be 1.5 gram per liter.

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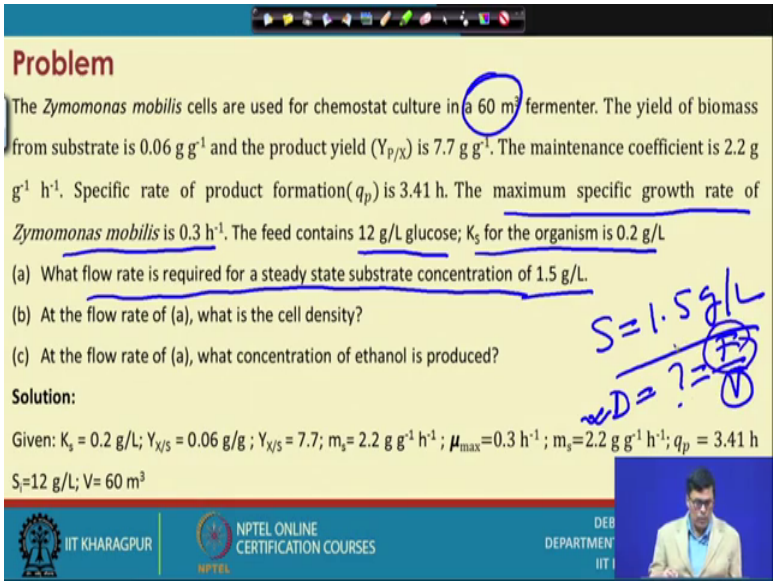

So, S value that will be under steady state condition is to the 1 gram per liter 1.5 gram that we shall have to find out. So, first we shall have to find out what dilution rate we have this value and if we know the dilution rate dilution rate equal to what F by V am I right. Now we know that the; our volume of the reactor. So, if you put the volume of the reactor we can if you know find out the dilution rate we can easily find out the flow rate.

So, this problem can be easily solve and then next part of the problem at what dilution rate at the at the dilution rate of a for will.

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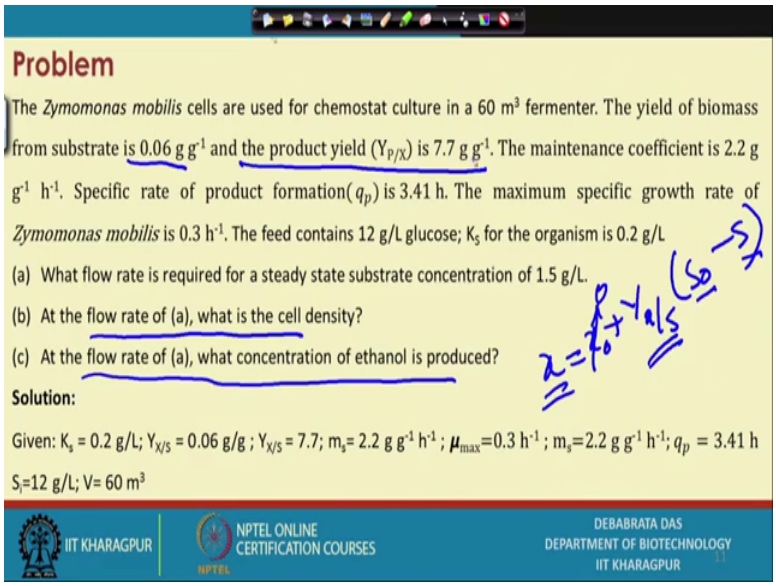

Be the cell density. So, X equal to what X equal to usually X 0 plus Y x S 0 minus S 0 minus S. So, here we have if we if we considered the field then this should be equal to 0; then Y x by s the cell will already given here is the you know value we know that is a hand then we can we can S, S also we know.

So, we can easily find out the cell density that; what flow rate the concentration of ethanol is. What at that flow rate? What will be a concentration of ethanol produce that we have given? The yield of the product that is ethanol yield 7.7 gram of ethanol per gram of cell mass though we can easily find out.

And I told you that whenever we try to solve any kind of problem, it is always a recommended that you should write all the parameters. So, here these are the parameters that has been given in please write it down.

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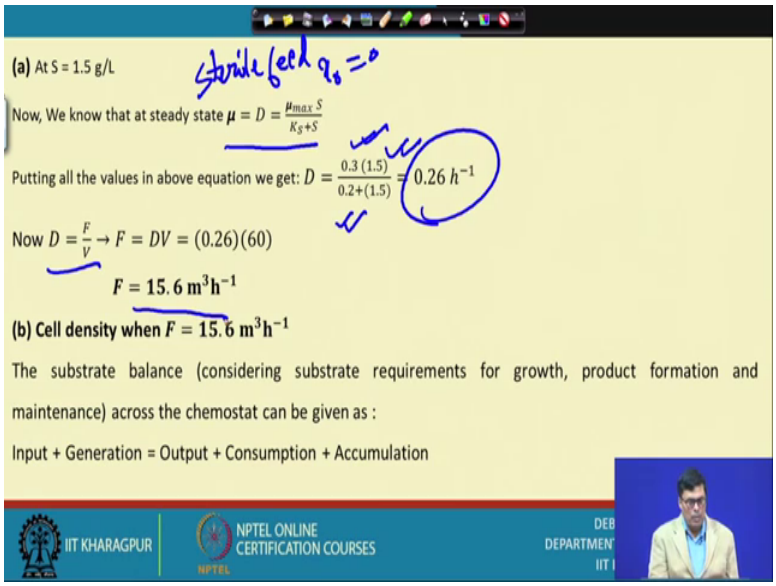

Then let us see I told you that; how to solve it? So, this is the equation that, we have then we have this is D equal to mu max. Under steady state condition and sterile feed sterile feed this sterile feed means X 0 equal to 0 then your mu equal to D then we can write mu max S Ks plus S am I right.

Now, in this the problems that will be all values are mu max value we know S value you know Ks value you know. We can find out the dilution rate. Now dilution rate equal to F by V and so, if f equal to D into V. So, your flow rate will be 15.6 cubic meter per hour. So, it is very easy to solve it now; once you find out that we shall have to find out the cell density; how we can find out the cell density?

Now if you do the substrate balance the rate of input may plus rate of generation of substrate equal to rate of output of the substrate plus rate of consumption of substrate will accumulation of substrate.

Now, at under steady state condition this will be 0 and in case of substrate balance, this will also generation will be equal to 0.

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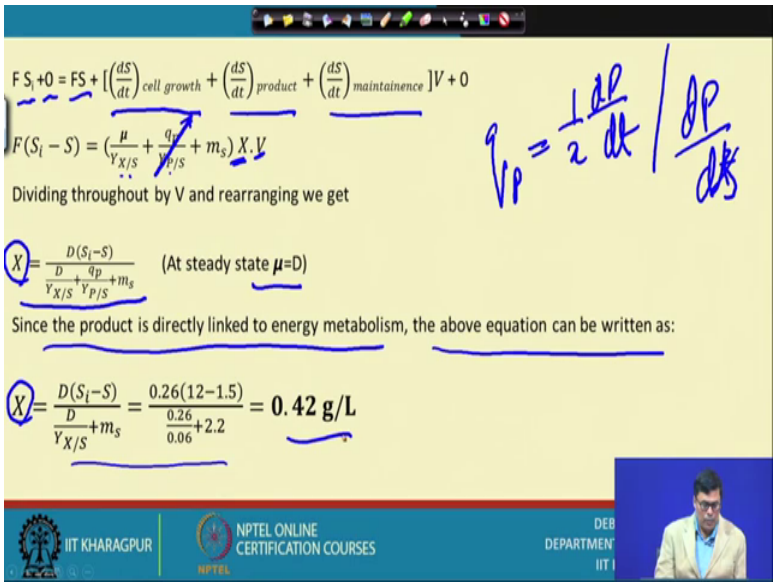

So, what we can write that that here? We can write this is equal to 0 and this is F S is the this is FI is the incoming initial substrate concentration. This is final substrate and dS by dt that is that is that is contributing for three different purpose. One is for cell growth another is product formation another is maintenance of the cells.

So, this can be written as mu by Yx by S qp by y p by s we have what is the qp I told you; qp is the specific rate of product formation DP by dt am I right. So, if you divide by DP by ds, then what you will get ds this ds the DP by DP will cancel then it will be ds by dt the specific rate of substrate conversion if you multiply by x and you will get the amount of substrate utilize for the product formation and if you consider multiply the volume consider whole volume.

So, if you consider this you can find out the equation for the X; X is the final cell mass concentration. So, then under steady state condition mu equal to D, if we assume that mu can be replaced by D and then equation would be like this then if we a since the product is directly link with energy metabolism that if we have the above equation.

Then we relate relater is like this we can assume that there is no product formation here then the and the mostly that the substrate goes for cell mass formation then the equation would be like this and we can find out the; what is the final cell mass concentration we can find it out?

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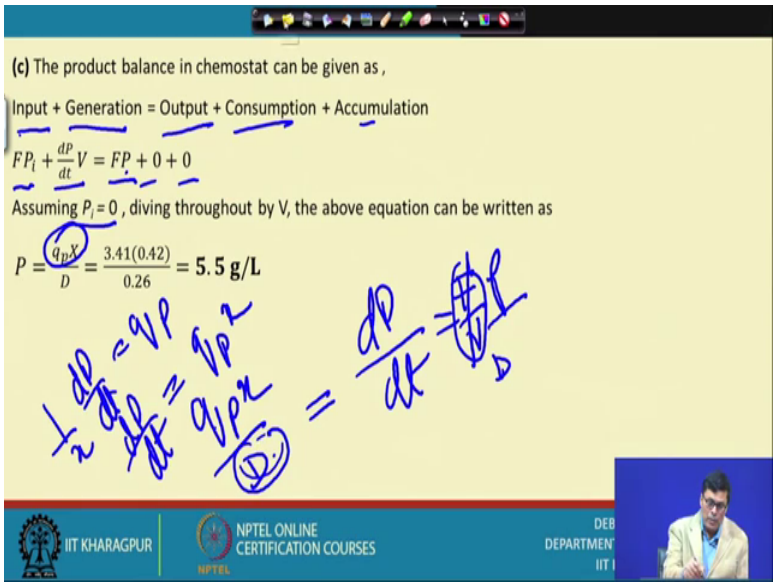

Now lastly; if you do the product balance say as similar to your substrate balance, we can have this equation rate of product input is F into pi the product generation product formation takes place DP by dt into V. This is the product output F by P and consumption will be 0 and also accumulation will be 0 under steady state condition. Then if we put pi equal to 0 divided both side by , then by V if you if you divide then what will get how you will get that the equation you will get that this equation I can write like this DP by dt equal to P am I right.

And DP by this is the this equation I can write qp into X by D D is the dilution rate, because if we if we divided by p this is P by P F by F by V is there. So, here F by V is the dilution rate though this. We can bring it here this D we can we can take it here. So, and what is qp is the specific rate of product formation DP by dt.

So, this is repeat we can we can easily multiply that we can then we can write this equation. Let the qp this. So, DP by this if it is the qp then I can write the DP by dt equal to qp into X. So, that is the, what is written and D will be coming from here and then we can we can come across this equation we can come across this equation and we can solve this and find out the product concentration. So, it is it is it is not very difficult.

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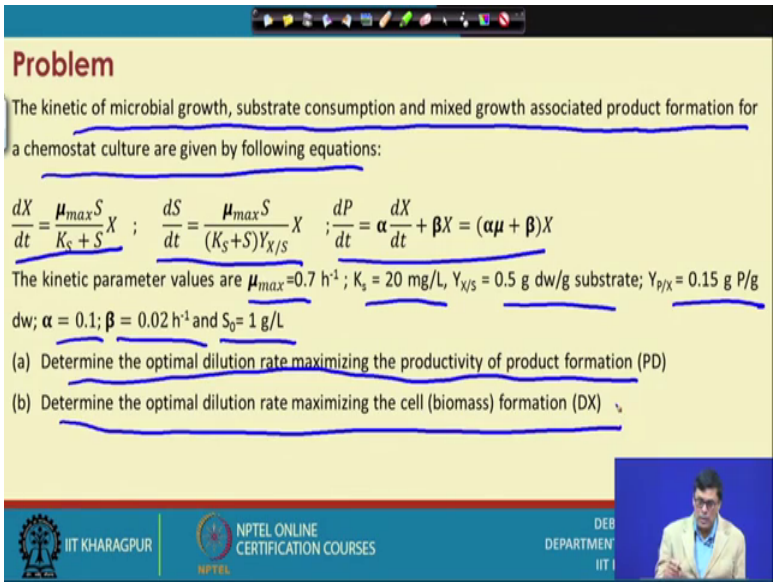

Now, last problem as I told you that this is with respect to luedeking Piret model. The kinetics of the microbial growth and substrate and mixed growth associated product formations of the chemostat of the equation this is the; we know this is the rate of cell mass formation rate of substrate digression rate of product formation with respect to luedeking piret model and this are the different data that is been given in this problem alpha value beta value S 0 value everything is given. The determine the optimal dilution rate for maximizing the product formation and determine the optimal dilution maximizing the cell mass formation.

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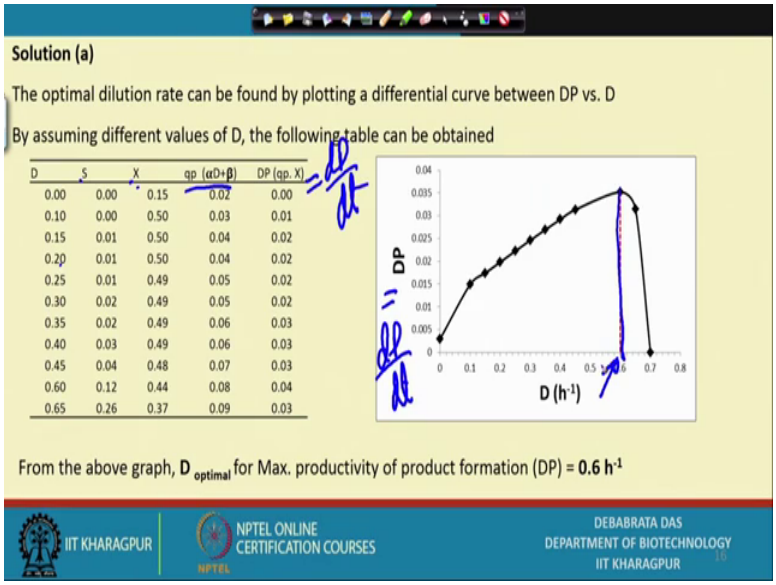

So, this can be solved very easily because what we can do we can we can write that that in this equation we know the DP equal to this is equal to DP by dx DP by dt equal to alpha dx by dt beta into x is we can write alpha mu plus I can take x common; then it will be 1 by x dx by dt that will mu. So, alpha mu. So, this will be alpha mu and plus beta into X. So, DP by dt I can write in this form.

So, in this equation we can every all values are given just we put the different dilution rate we assume different dilution rate because D max value is given. So, we can assume the D value less than that 0.1, 0.15, 0.2, 0.3. So, respective S and S and X value we can calculate and we can calculate the qp value that then we can find out the D into P value that is the qp into x that is this is equal to DP by dt am I right.

Now, if you plot this is equal to DP by dt now if you plot DP by dt versus D. So, will be having this kind of plot and this dilution rate you will get the maximum rate of product formation. We can easily find out optimum dilution rate at which you will get the maximum rate of product formation.

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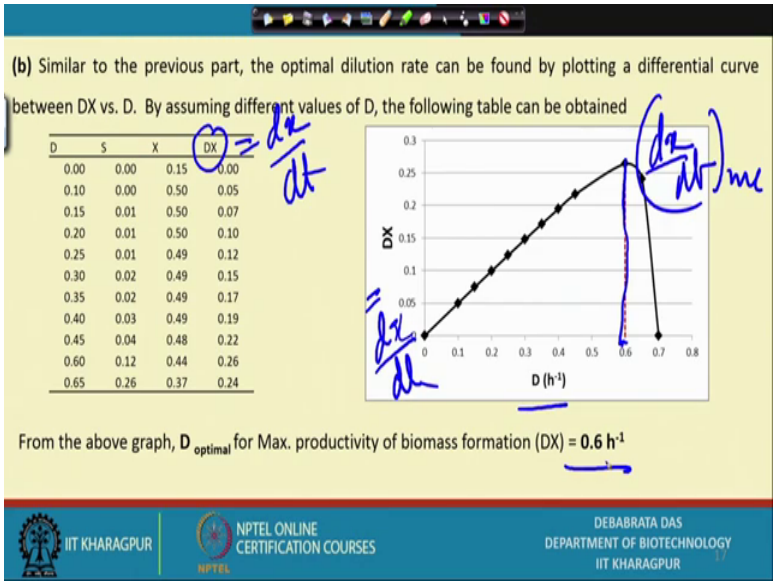

Now, in case of cell mass formation that we know this we have done before also that that here also we can find out the different dilution rate what is the S value and X value we have already calculate and if we know the X value we can multiply by dnx dnx by what is this dx by dt am I right.

So, we if we if we this is equal to dx by dt. Now if you if you plot with respect to D; though we also you will get a dilution rate when you will get the maximum value of dx by dt is maximum. So, that is here also, but. So, both we have seen both the product formation and the cell mass formation we required maximum we required the as the same dilution at 12 point hour inverse.

So, in this particular lecture I try to discuss that; critical issue that critical issue with respect to that how batch data can be use for extrapolating to find out the cell mass concentration in a chemostat and how the cascade reactor? The cell mass concentration of the cascade reactor can be determine graphically; how diauxic growth can be analyze and then we find out we try to solve some kind of chemostat process and finally, we discuss under at what dilution rate you will get the maximum product formation and also maximum rate of cell mass formation.

Thank you very much.