Course on Industrial Biotechnology
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Lecture 17
Life Cycle Of The Microbial Cell, Microbial Growth Kinetic, Product Formation And Substrate Degration (Contd.)

Let me welcome back here again in my course industrial biotechnology. Now I was discussing about the microbial growth kinetics product formation and substrate degradation.

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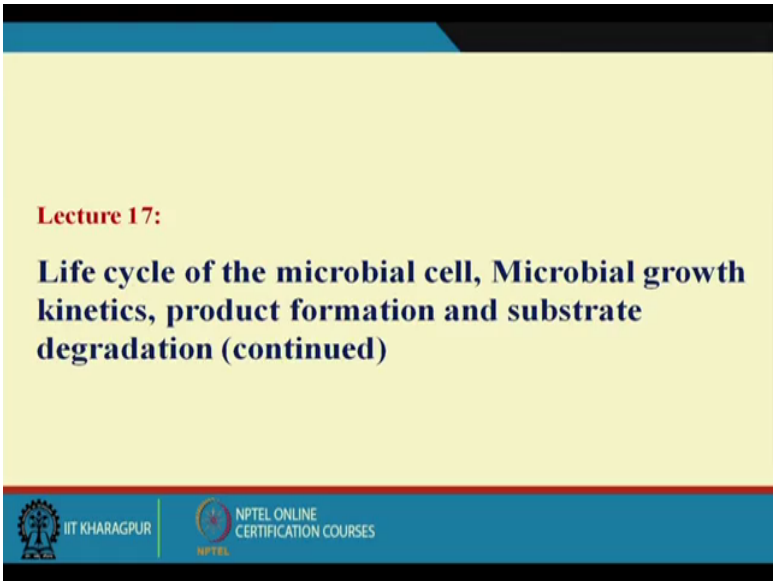

In the last class I try to find out that that what is the role of for life cycle for the growth of the cells. I told you when whenever we do the inauguration of the because different and the growth cycle we have different phases and different phase has the difference significance. Lack Phase is considered as a actualization phase growth which is considered.

The Lock Phase is considered as a growth phase where the organisms are active and in stationary phase is considered the starvation phase where most of the secondary metabolite formation take place. Now when you do do the inauguration of the cell (())(1:19) to do the inauguration of the cell.

In the either mid lock phase to get lock phase. Just to ensure the organisms are active. Now um we used the Michaels menten equation. I showed you the menten equation so for the study the growth of the organism. So similar to Michaels menten equation only we come

across the is which is considered as a growth limiting substrate and the growth limiting substrate might be anything because media companies is n number of chemicals.

It maybe carbon source, it may be nitrogen source, it may be vitamins, it may be minerals so anything might be the growth limiting substrate provided the growth is if viewed he from if it changes with respect to change of concentration of the particular component present in the reaction mixture then and only then we got growth limiting substrate. Then we come across the batch process and the.

And the chemostat process. Batch process is the process through which we can have this we can find out the life cycle of the cells but major disadvantage of the batch processes that we cannot hold particular phase of growth for infinite period of time. As the time passes on one phase which which what to the other phases. But in the chemostat process we find that under ()((2:47)) condition.

And and at the steady state steady state condition meu equals to D, so by controlling dilution rate it is possible to maintain a particular phase of growth in the in the fermentation block. So we can we supposed particularly Bakers is fermentation process we need more cell mass production so literally that rate of cell mass production report in the in the growth phase but in case a batch process.

We cannot hold the phase for longer period of time but in the in case of chemostat process we can continue this growth Phase for infinite period of time. I think and how to find out how to calculate this this growth of the organism? How to calculate the softer degradation? I show in the next class.

Now now this particular lecture I try to concentrate that we will come across the Dmax and D wash out which is very important as well chemostat operation is concern. Then with this can be overcome by using either by mobilization of the cell or by the the recycling the cell mass to the reactor and how we can do that? So this this year I am going to highlight in my lecture.

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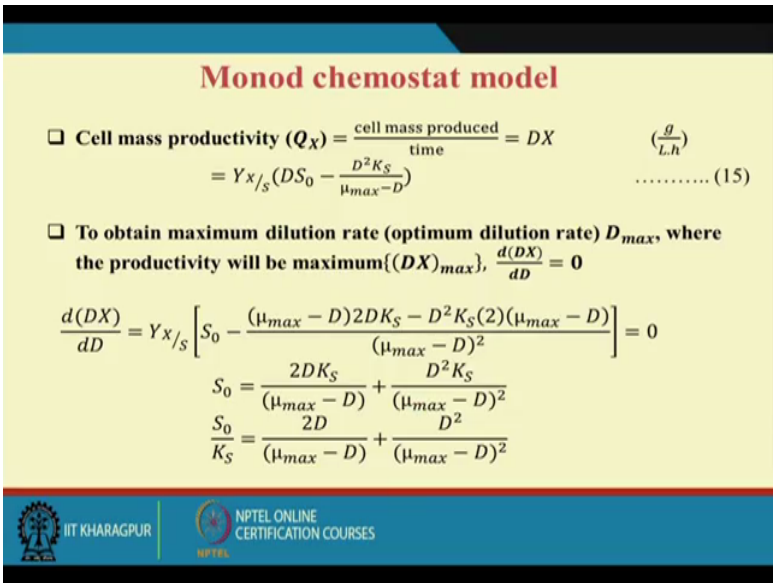

Let me start with that, first let me show you how you can calculate the D Max?

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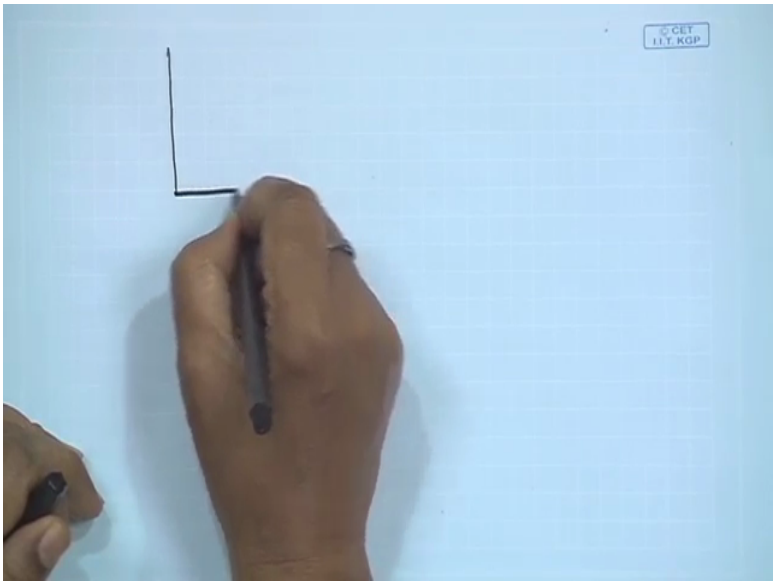

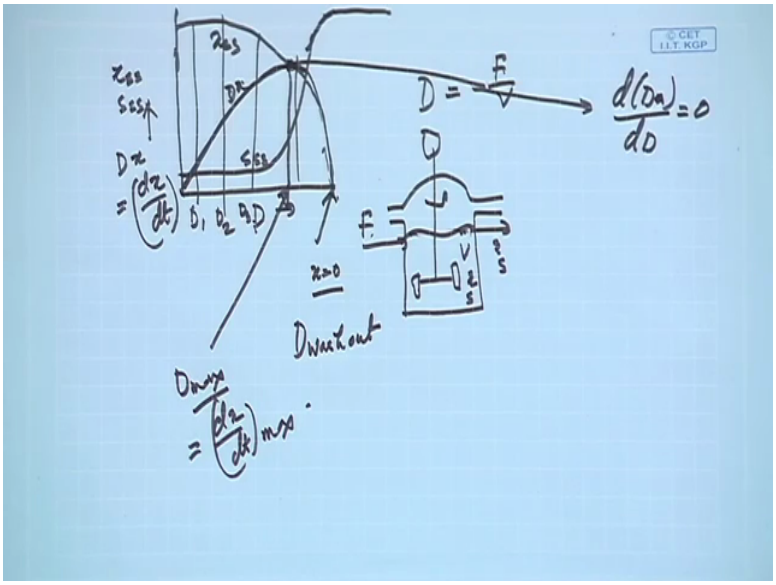

D Max is considered as a that maximum the dilution rate at which we will get the maximum rate of cell mass production. This is equal to I showed you before that D into X is nothing but DX by DT what is DX by DT? Is the rate of growth of the cells. Now this will be like this is go like this and this will come down like this. Now at the same time we can have the plot for X. X is is the steady state cell mass concentration.

The state State substrate concentration and steady state concentration will be like this it will go like this then it'll be constant and then your your cell mass concentration will will come back here. This is Xss and this is Sss. So this is very important because because as as your dilution rate increases because this is the different dilution rate. This is D1, this is D2, this is D3. Now I told you dilution it is equal to what F by V.

So in case of any kind of Chemostat suppose this is this is any chemostat, if you pass this, this is F and this is V. So we can change the value of D if we simple increase the value V is constant. The volume of the reactor the volume of the working volume of the reactor is constant. So if you want to change the value of D we can change the value of F when you change the value is changed.

At different dilution rate I told you that when you change the value of a after infinite time will get the steady state condition. What is steady state condition? When the cell mass condition will remain unaltered. When the substrate concentration cell mass concentration they will unchanged with respect to time that is called steady state condition. T at the different dilution rate you get the different steady state condition.

So this is exactly what is happening, as the dilution rate increases, your steady state cell mass concentration will finally this is a crucial point where X equal to 0 X equal to 0. No cell is present in the reactor. If there is no cell present in the reactor there will be no reactions. Because cell mass is usually responsible for carrying out the reaction and this is considered as D washout.

D washout means that at the dilution rate there is no cell present in the reactor. This is the D washout and hear you give DX by DT this is DX by DT with respect to time increase and then decrease. Now here we have a point and this point we considered as the D-max what is Dmax? Dmax is the dilution rate when DX by DT equal to maximum. that means what we can we can write.

When when at this particular point I can write at this particular point D DX by DT this should be equal to zero. Because this is D this is the DX this is DX and D DX by DT this this is Evan Plato so I can get there at the Dmax this is the condition that we have. Now now let me come here.

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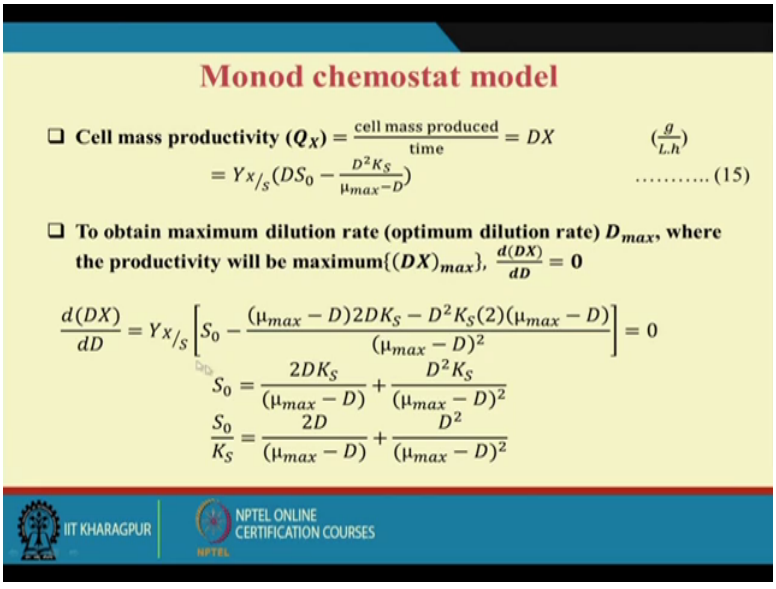

That here we can write that DX by DT we can write like this.

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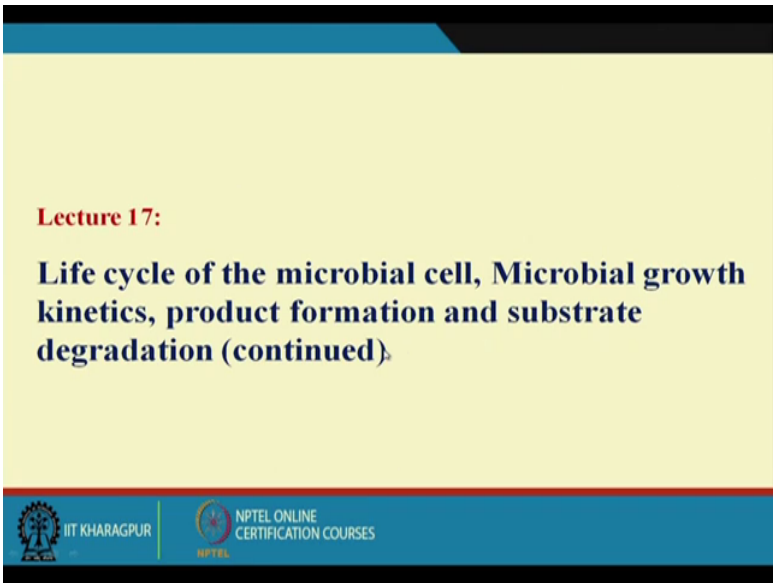

Because we have we have we have seen that with this is the expression that we have then S0 we can write like this is the way we can write the like this.

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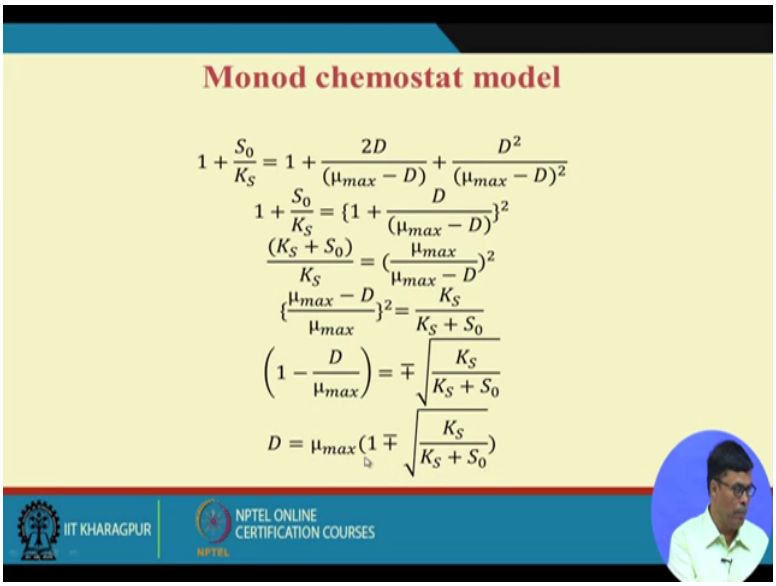

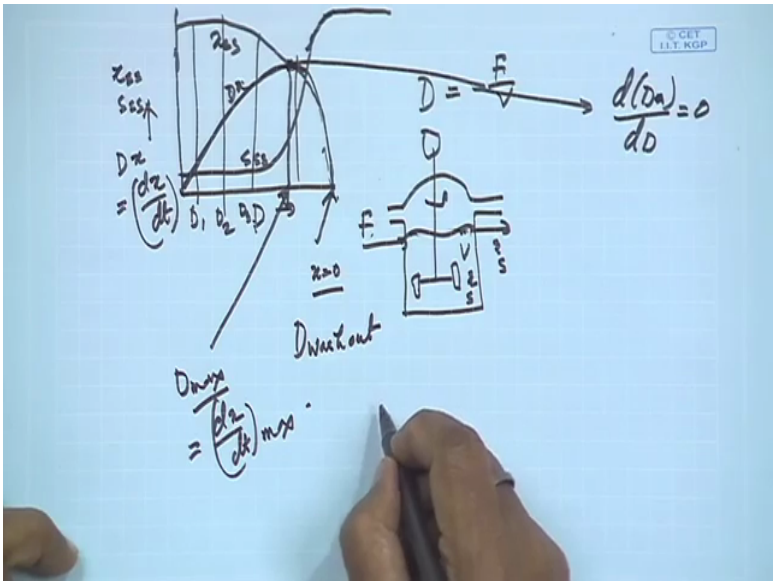

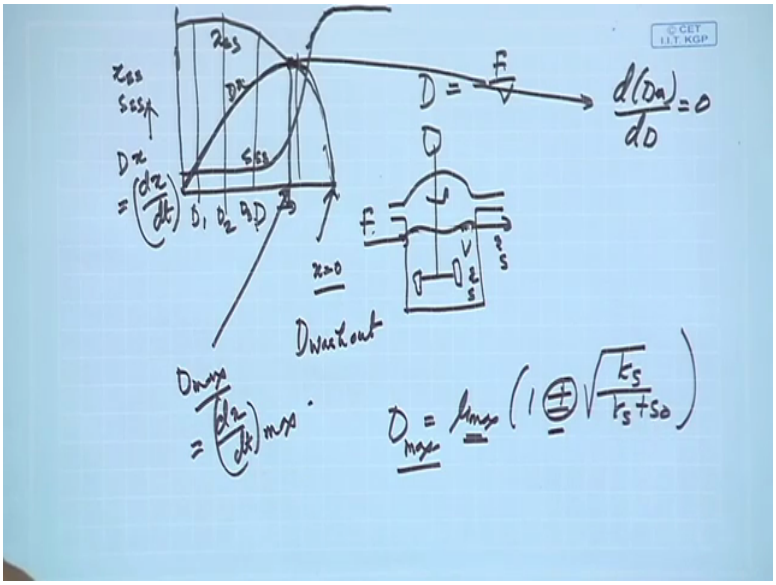

Ultimately we will come now come this equation and this equation is what this is D equal to mew Max one plus minus Root KS + KS + S0. Now one thing I want to find out that you are that this is this is the Dmax value this is the D max value we can calculate like this. Now the Dmax value always should be less than mue Max value. So it cannot be plus it will be always negative.

So usually the D max value will be 1 - Root Ks ()((9:12)) then we derive this plus minus but actually they cannot be possible is always should be less than mu Max minus KS by S0.

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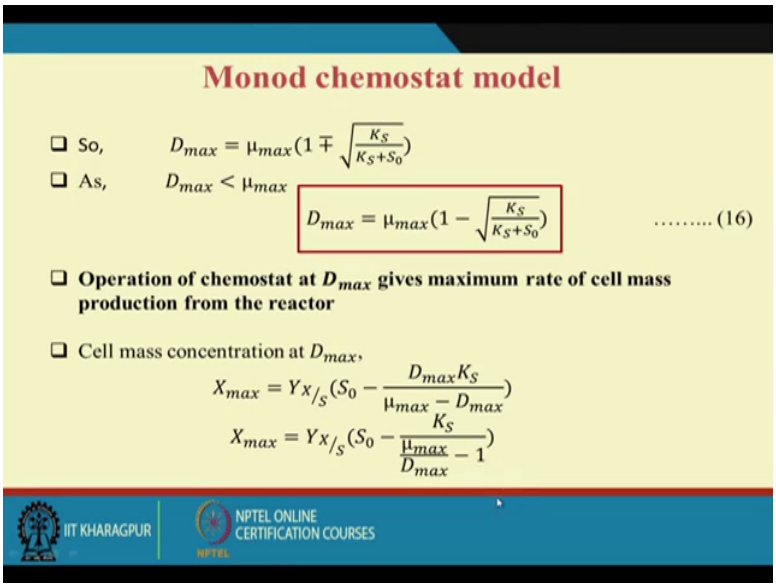

Now here Dmax equal to like this. But since D max is much less than mu max so we can write it Dmax equal to meu max 1 minus root KS by KS plus S0. Now that operation of the chemostat at Dmax is the maximum rate of cell mass production from the reactor. The cell mass now how we can find out what is the maximum rate of growth of the cells? Suppose what what you can we can do in a chemostat?

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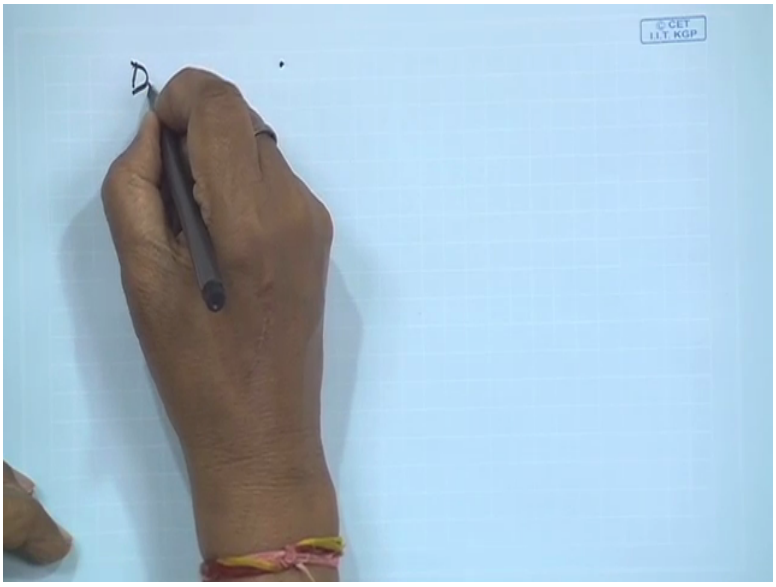

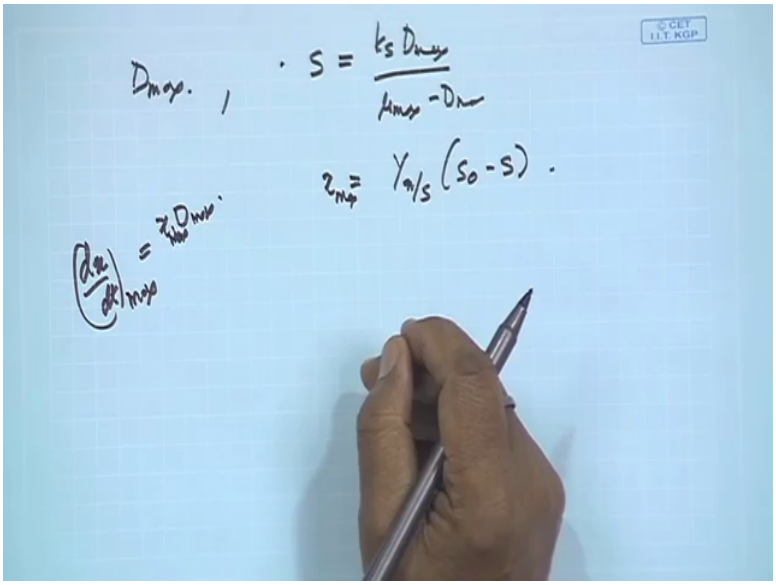

If you if you if you find out the D Max value then you can find our what is the S value? S value is What S value is what we can write KS Dmax meu max minus D Max. I can easily find out this this this value. We can and similarly I can find out the value of X. This is X equal to this is the maximum value of X max is equal to what YX by S into S0 minus S. So this is now X X max.

What is the when rate of growth of the cell rate of production of cell is maximum when X into DX Max into D max. If you multiply that you can we have we have come across like this. Now now if we can we can we can do the calculation in other way also.

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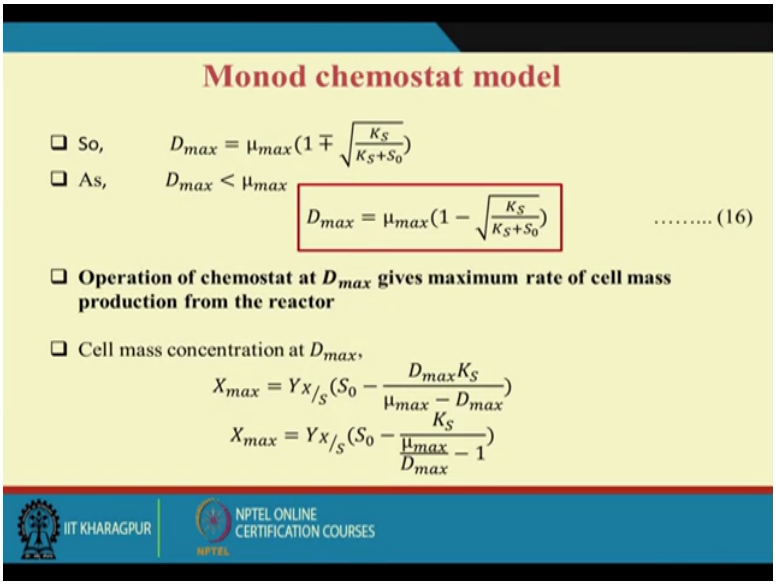

Suppose this we we we calculated the cell mass production this is this is this is X X X X Xmax equal to YX by S this is the S0 minus this one. That is the substrate concentration the that is the rate of substance that you consume that you can find out the value that form that we can calculate this this one and finally we come across the Xmax value is this one.

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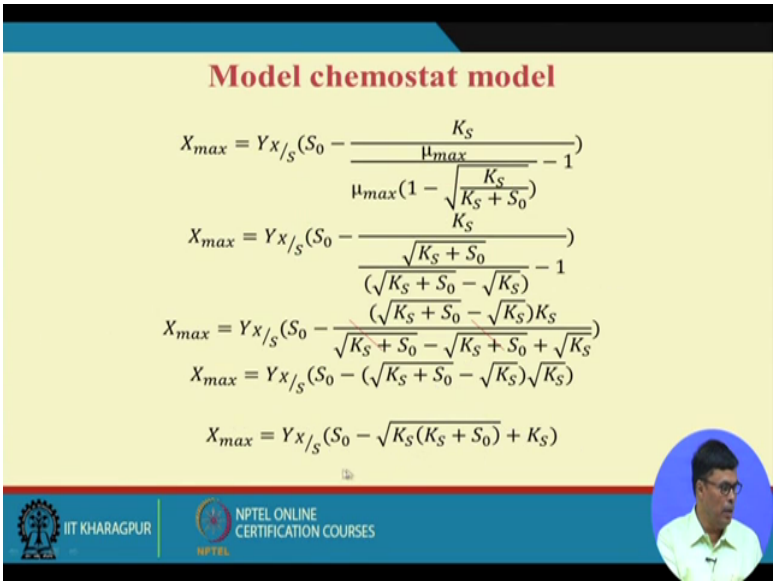

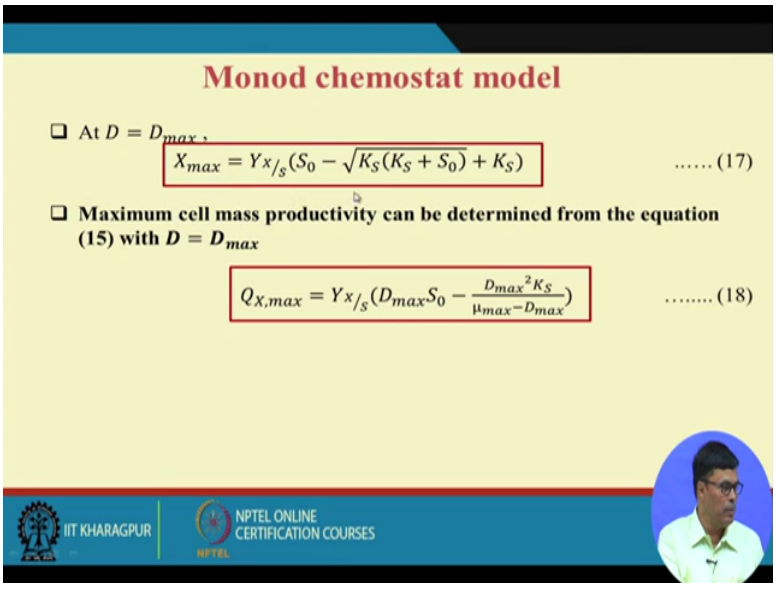

And Dmax we find out this equation. We derive this equation equal to Xmax equal to YX by S0 root over KS KS KS into KS plus S0 plus KS. The maximum cell productivity can be determined by the equation D equal to D max. Now we multiply D with the D max we will get this equation. This is then equal to this is the maximum rate of production will be YX by S into D max into this that we have in the previous equation that can we can write in the 15 equation.

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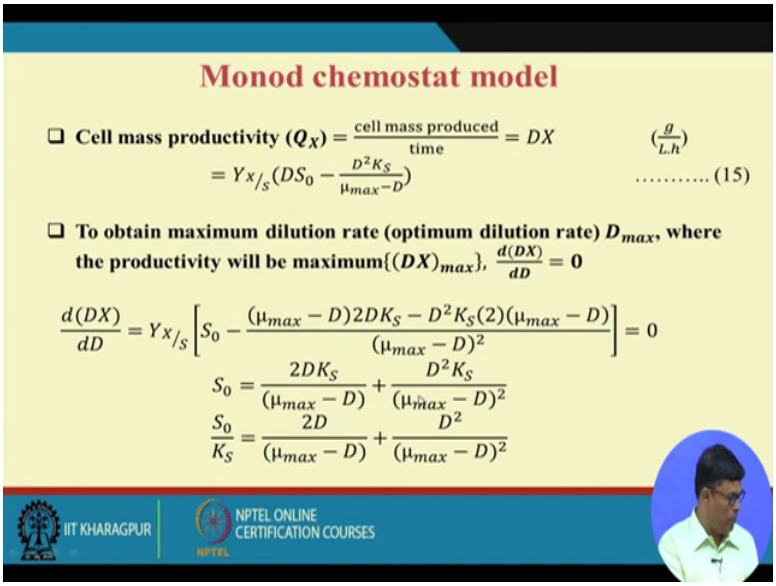

So in the 15 equation this equation we can put this value we can put this value in D Max V equal to D max. We can have this equation this is we can write this.

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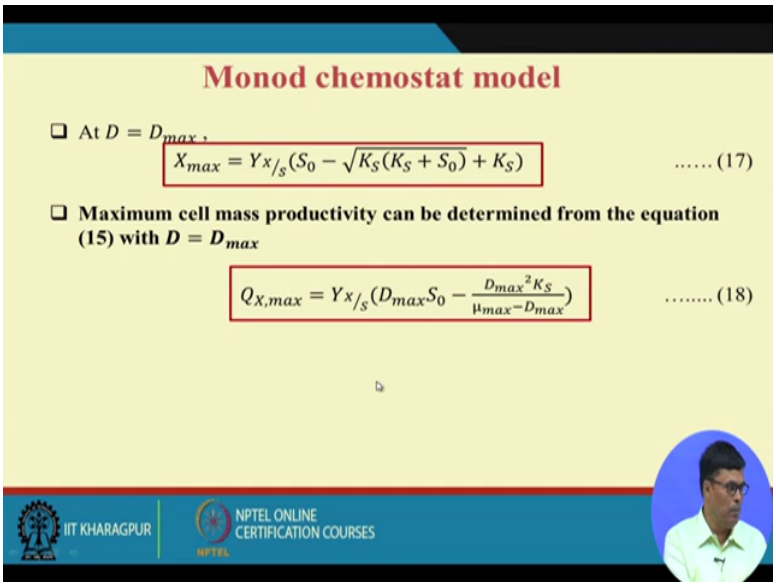

We can find out the maximum cell mass production.

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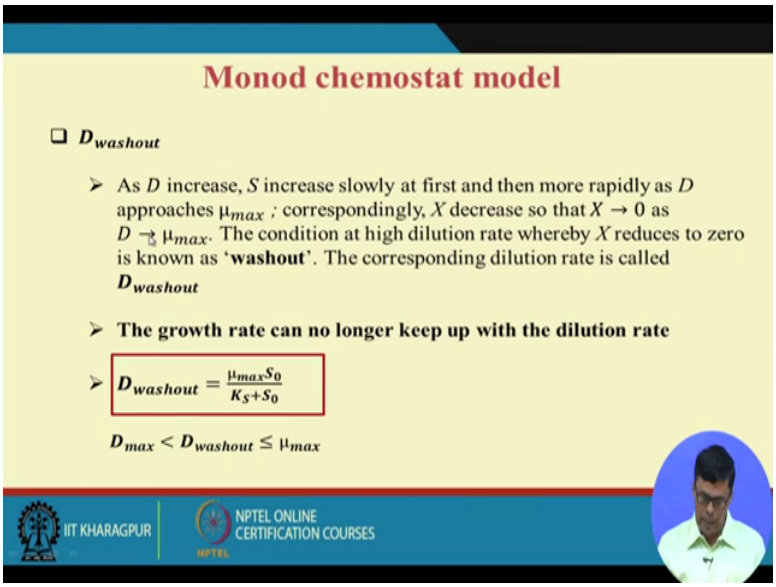

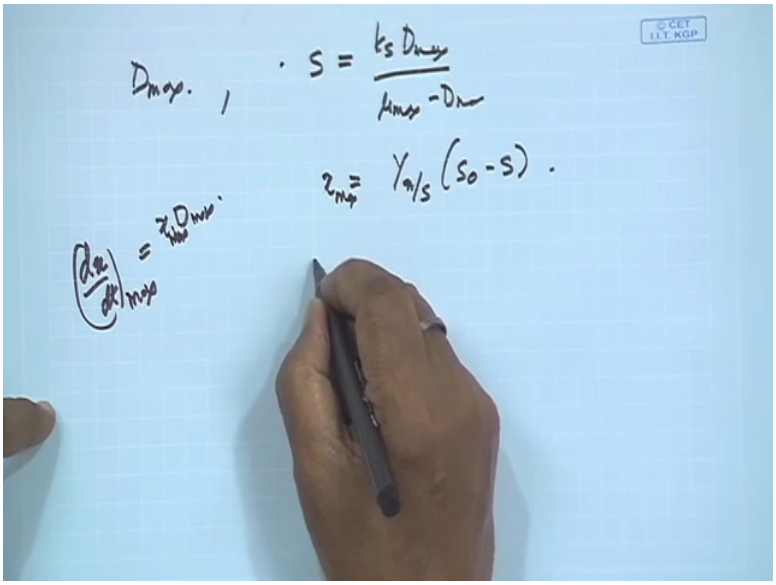

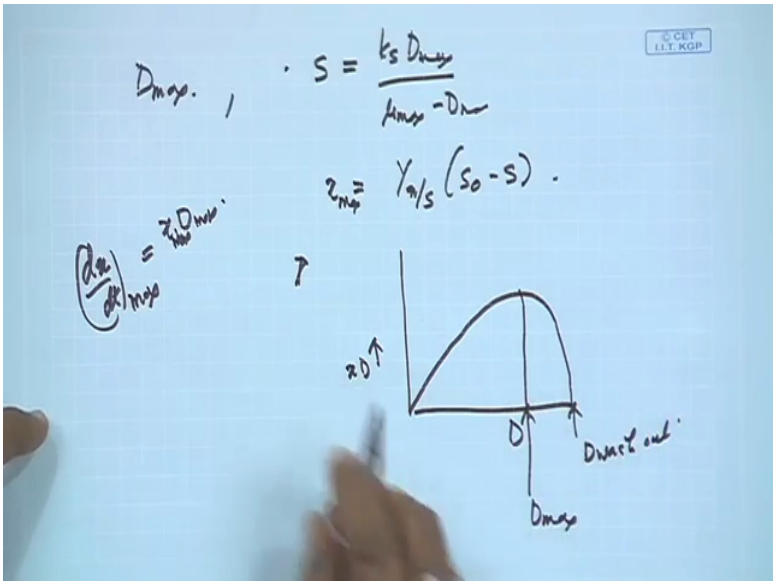

Now in case of Chemostat process major drawback that we have the Dwashout because that the wash out if you if you look at it I can show it like this that if you have X into D related with correlated with this one.

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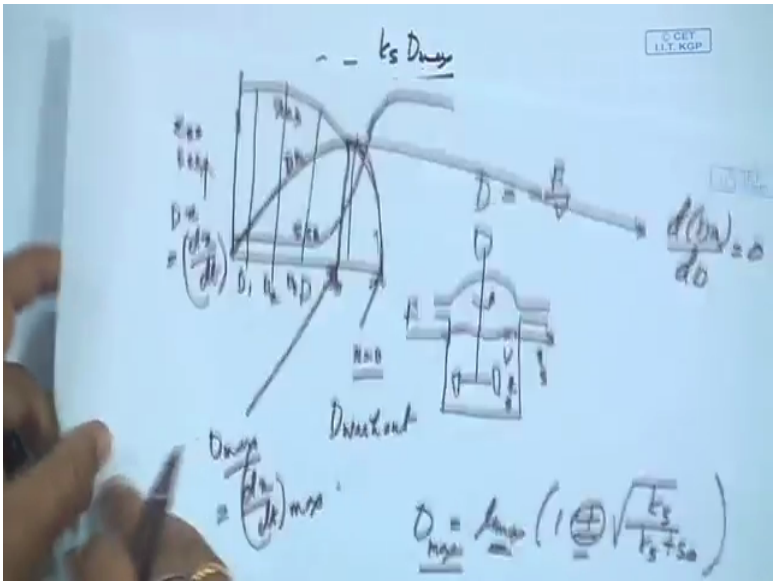

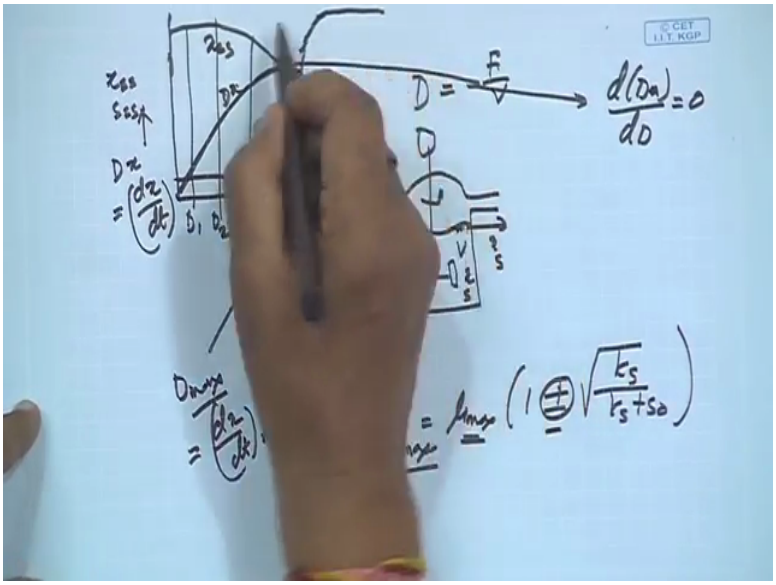
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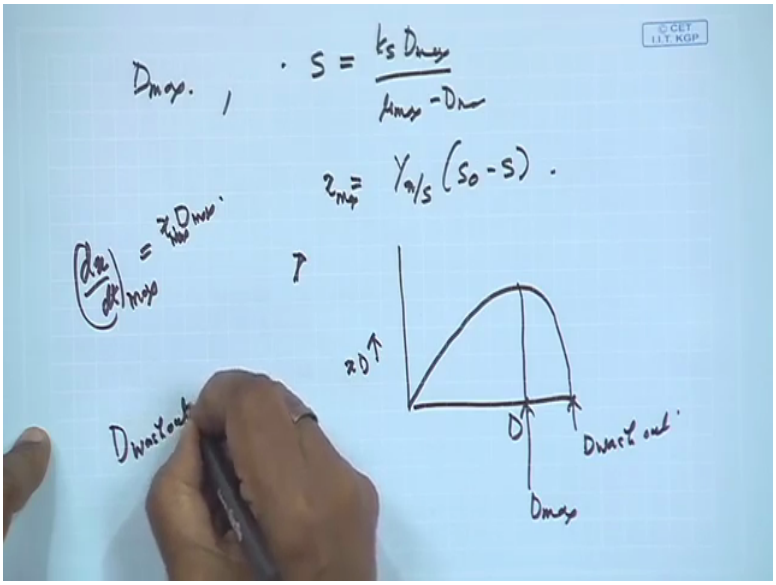
$\frac{d(D\alpha)}{dD} = 0$
D_{max}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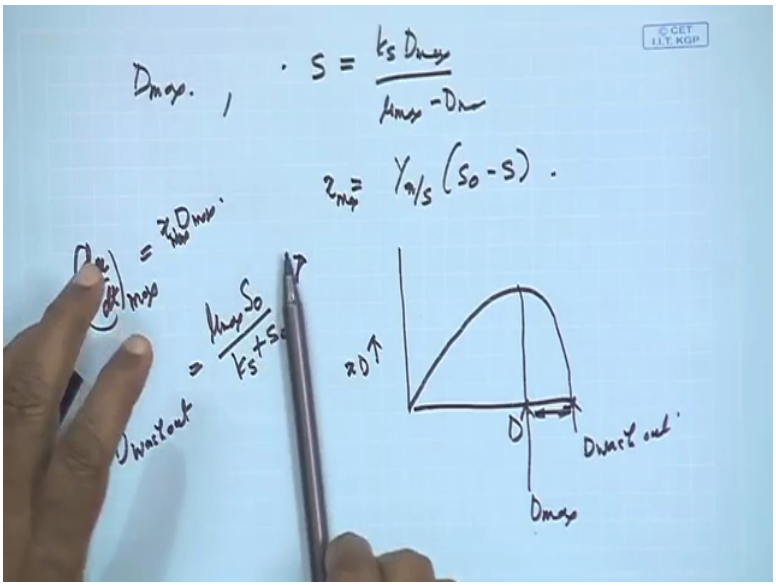
D_{max}.
$S = \frac{k_s D_{max}}{\mu_{max} - D}$
$r_{max} = Y_{x/s}(S_0 - S)$.
$\left(\frac{dx}{dt}\right)_{max} = \mu_{max} D_{max}$.
$D_{washout}$
$xD \uparrow$
D
D_{max}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D_{max}.
$S = \frac{k_s D_{max}}{\mu_{max} - D}$
$r_{max} = Y_{x/s}(S_0 - S)$.
$\left(\frac{dx}{dt}\right)_{max} = \mu_{max} D_{max}$.
$= \frac{\mu_{max} S_0}{k_s + S_0}$
$D_{washout}$
$xD \uparrow$
D
D_{max}

This is like this. So you have two two points we have here you have D max value, here you have D wash out value D wash out. Now as D wash out value I showed you it before that here here I have showed you that at D washout value this this X will be zero and if X will be zero this corresponding value will be X0. That is that is corresponding point will be S zero so if it is S0 then what what will be the D washout value?

D washout value D equal to ()((13:36)) steady state condition I can write mew max S will be S0 plus KS plus S0. So since there is no cell when reaction takes place so S is considered as S0 and if you put the value of S0, you can easily calculate that. So we have we have the expression with us Dmax value and they are very close to each other and in the industry.

Here I want to mention that in the industry when you operate a chemostat process that we don't have different forms for the operation of different product ()((14:15)). We use for maintaining the different product and how we maintain the different product? Just just opening the valve because we have seen in the wash basin when you open your valve then you'll be more water flow tech plus.

If you close the wall you will find less water fall. So we can control the flow of water with the help of valve. In the in the industry that that flow rate is controlling by the with the help of valve. Now by mistake with operated this is usually done by the operator increase the valve then there is a possibility that D max will switch what to the Dwashout that is the major drawback that we have with the chemostat process.

And then question comes that what is the physical significance of the D washout? Why the D wash out take place? The reason is that one reason I can tell you that I am I talked about the generation time. What is the generation time? The time required for the cell division. Now 1 by D is what? 1 by D is the hydraulic retention time. Hydraulic retention time is how long you allow the liquid inside the reactor.

Now suppose the cell has some generation time. Suppose this whatever bacterial cell usually they have the generation time 15 to 20 minutes. Now if you if you allow the liquid less than 15 minutes or so maybe 10 minutes then what will happen you will not allow the bacteria to grow. Before it grow you are taking out the bacteria. So you are not getting any any cell in the reactor D washout. So the another way I can explain the situation this is suppose this is chemostat process.

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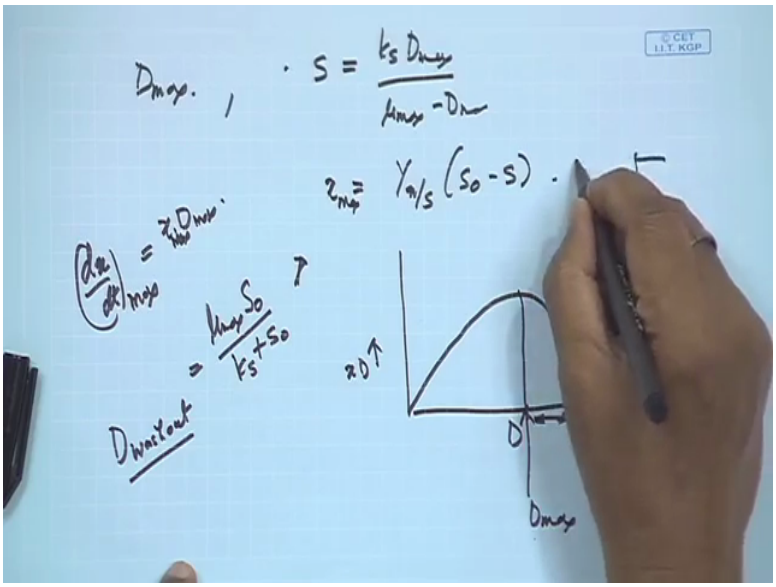

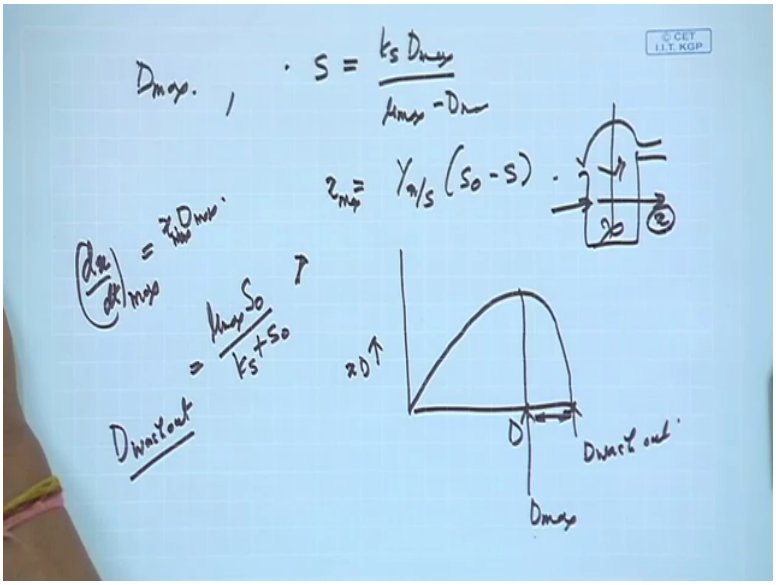

Now now now now if you operate the chemostat process like this now now here I told you that there is in the major drawback of the chemostat process is that always there is a cell mass loss on the reactor. If the rate of cell mass that is going out of the reactor is more as compare to rate of growth of the cell. Then what will happen? The time will come when there is no cell present in the reactor. That is alsothet is why we come across the D washout in this reactor. So we are we should be very careful.

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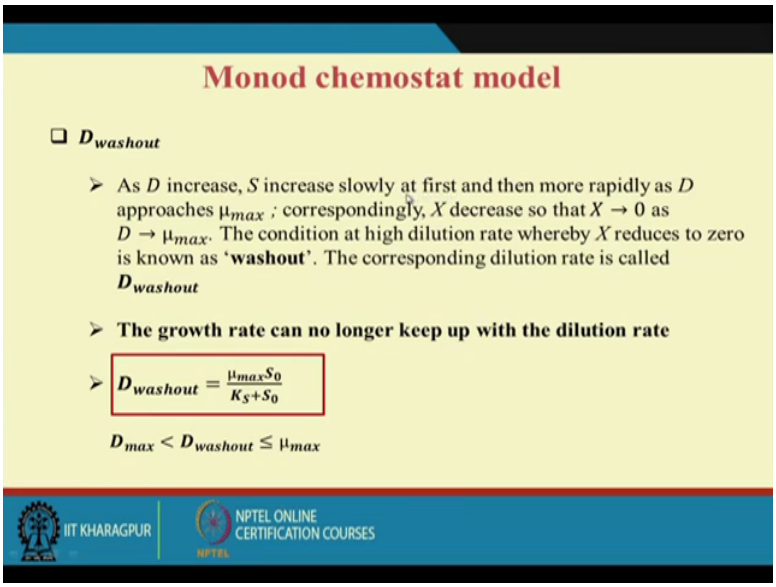

We mention here as the D increases S also increases slowly at first and then more more rapidly as D approaches mu max corresponding S decreases as X tends to 0. So this D wash out is equal to and the correlation between the D Max, D wash out and mu max is like this. Dmax is less than D wash out and D washout is less or equal to mu max.

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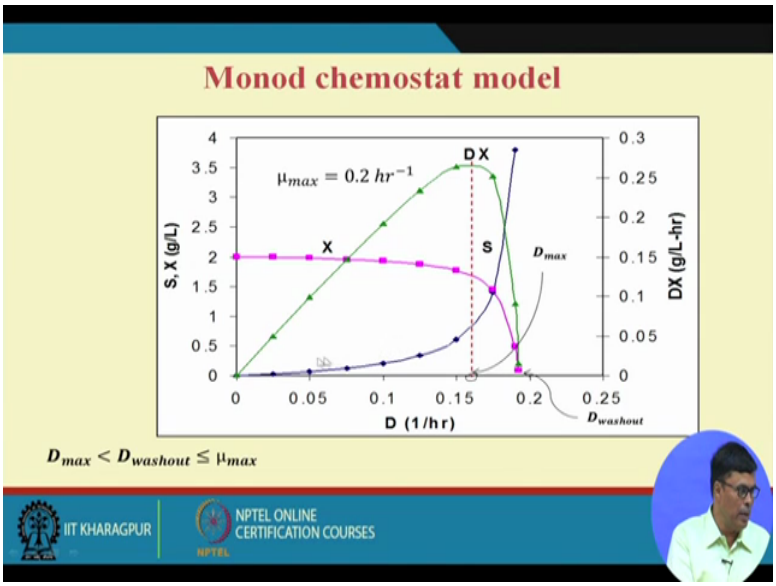

Now ()((17:17)) that you know we can we can we can graphically I can explain like this this is DX that is like this and this is the typical one particular fermentation process there we have tried to find out this where mu Max value is point 2. So here you see that D Max is around this point 15 and where D wash out is then less than point 2.

You can see that is not exactly point 2. So it is usually the D Max is higher than D washout value. So you can you easily find out and they are very close point 15 and this is about to less than point 2. So this is very close. So even little increased in flow rate there is it possible we can meet the situation.

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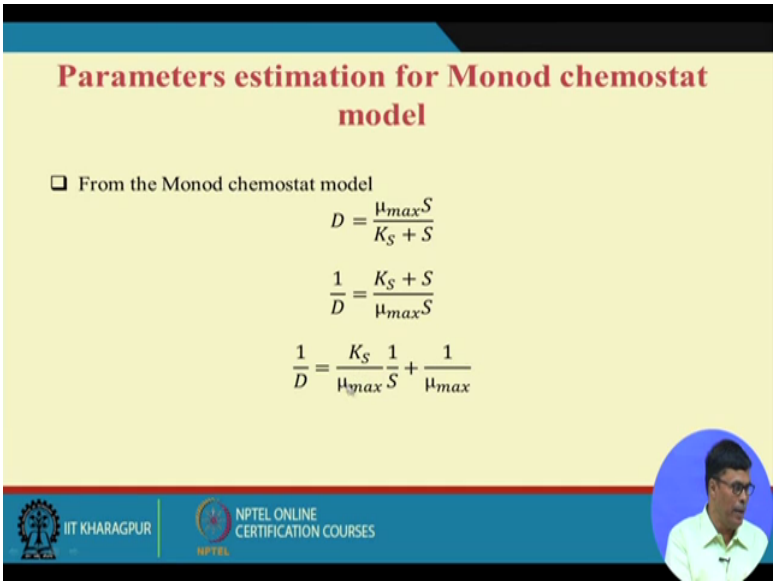

And now they do this how we can find out that.

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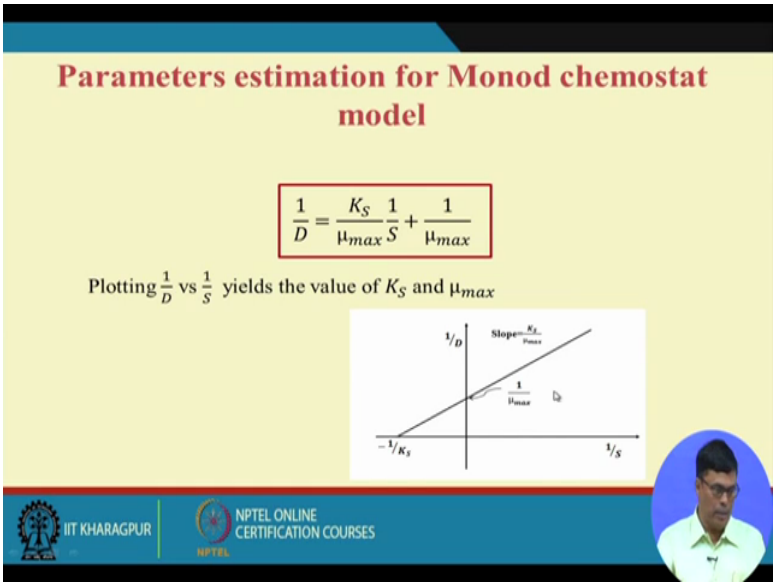

Now this kinetic constant of this of this mu max and KS we can determine the continuous process also. If we plot 1 by the D buses 1 by S we can have this line of ()((18:31)) and from

the slope we can get the value of KS by Mu max and intercept we can find out. So this constant value we can find out both by operating the chemostat as well as the batch process.

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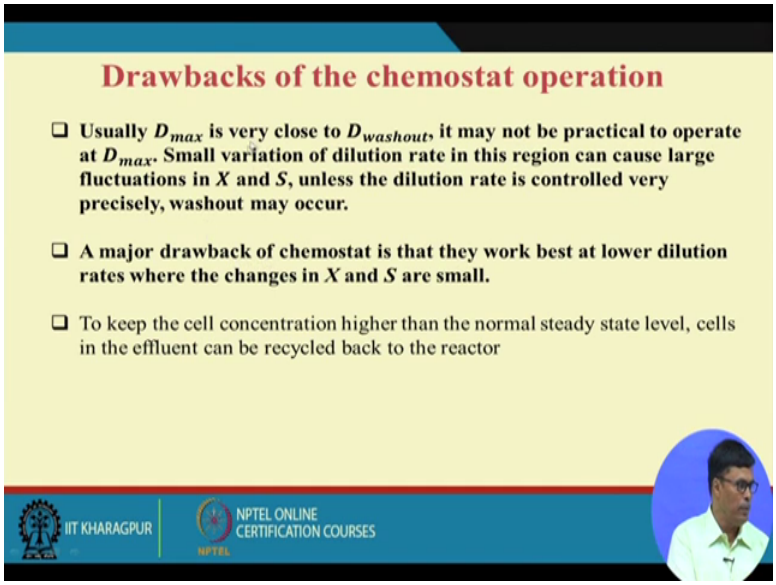

So the drawbacks of the batch process as I mentioned that mainly the D washout problem that we have and how it can be ()((18:56)) it it can be ()((18:59)) by two ways either you recycle the cell mass to the reactant whatever the excessive that is going out of the reactor you recycle back. The same cell to the reactor and other otherwise cell on the solid Matrix no cell out cell washout the reaction take place.

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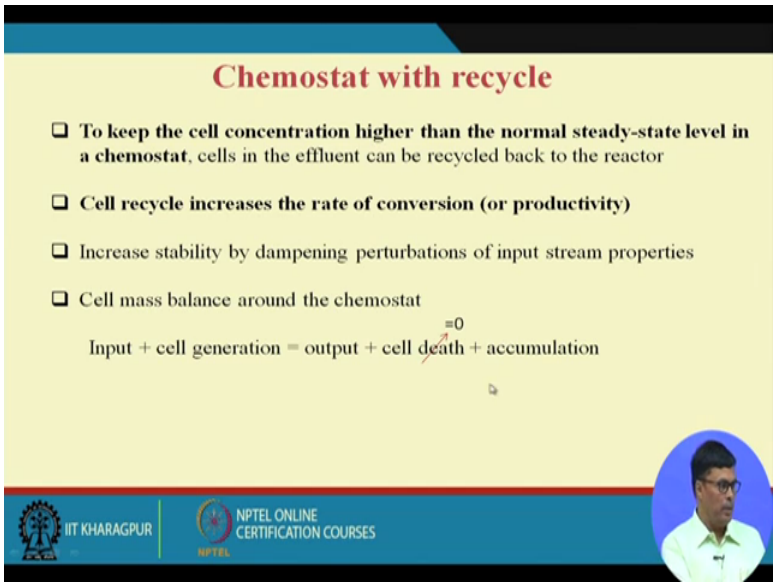

Now and let me discuss that how you can analyse the chemostat cell mass recycling because the major drawback that we have with the chemostat that can be can be overcome by recycling and this is adopted by the different industry. I can I can give the example of activated sludge process. Activated sludge process is largely used by the industry for wastewater treatment process because this is easily operated.

And here this is activated sludge process is nothing but ()((19:52)) cell mass recycling. The cell mass recycling increase the rate of conversion and increase the stability by dampening perturbation like this. So we can we can we can write the balanced equation is the inputs alienation output so it will be assumed to be zero and Accommodation.

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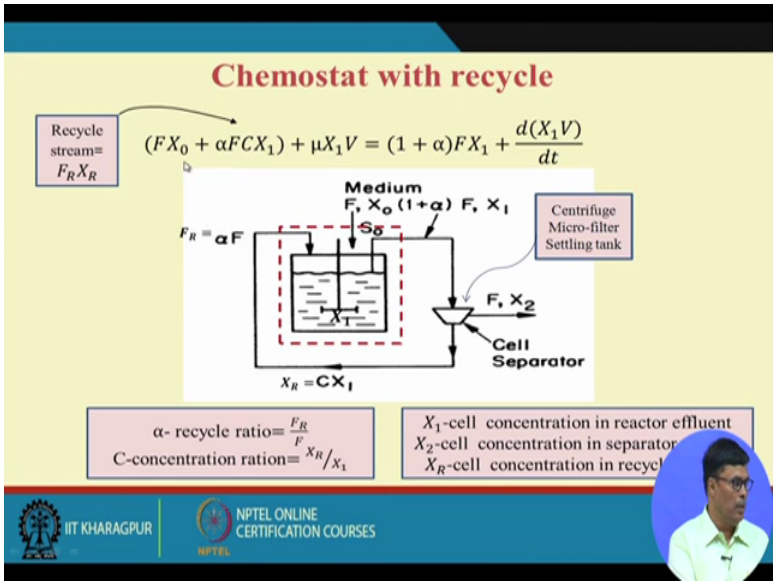

This is this is the equation I can write and alpha is the recycle ratio. Recycle ratio means I told you that what you do that here whatever the cell mass is going out with a pass through cell separator a part of the cell will recycle back to the reactor and part of the cell you going out of the reactor what do you call cell mass wasting from the reactor. The and here we can take out the supernatant here another way we can take a separator.

So if thet recycle will be the flow rate, if this is the recycle flow rate and if improve flow rate then f is considered as alpha. What is considered as the recycle ratio and XR is the second cell mass concentration. This is the cell separator. It separate the cell how easy easiest way of cell separation in the sedimentation another way is by centrifugation also you can separate the cell.

So you get the concentrated cell and peace with this is concentration with the XR and XR by the X X1. That is that is going out here. This is output cell mass concentration we can assumed to be see. Now obviously the XR XR and X X1 that should be always greater than one because under no circumstances it is less than 1 because XR is the second cell mass concentration and this is the suspended cell mass concentration.

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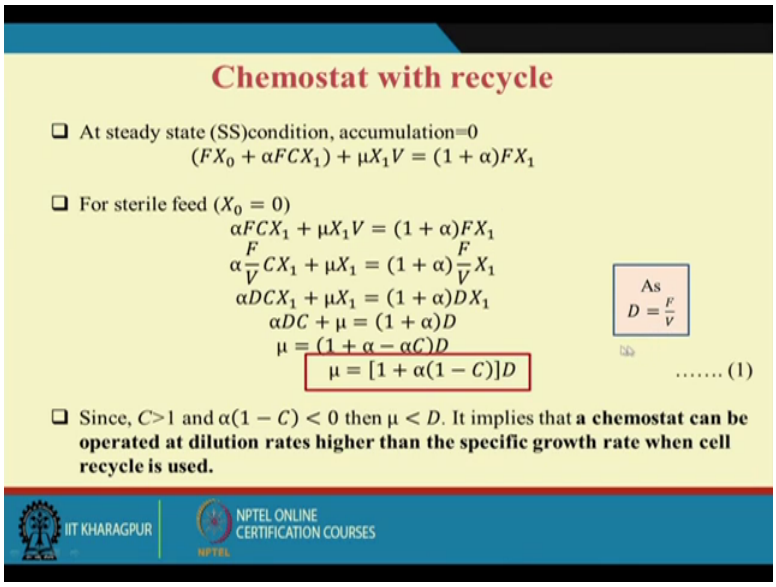

Now and we we write this balance equation the we try to analyse this equation and we come across the equation mu equal to 1 plus Alpha 1 minus C into D. This is very important because C as I told you C value always greater than one if it is greater than 1 then what will happen? This will be negative and if this this is the recycling ratio and this one minus negative will be less than 1 and so D will be mw Divided by fraction.

It can be operated at the higher higher growth rate that can be operated employees that chemostat can be operated at the dilution higher than the specific growth rate of the cells because I can show you that in case to steady state condition that mew equal to D.

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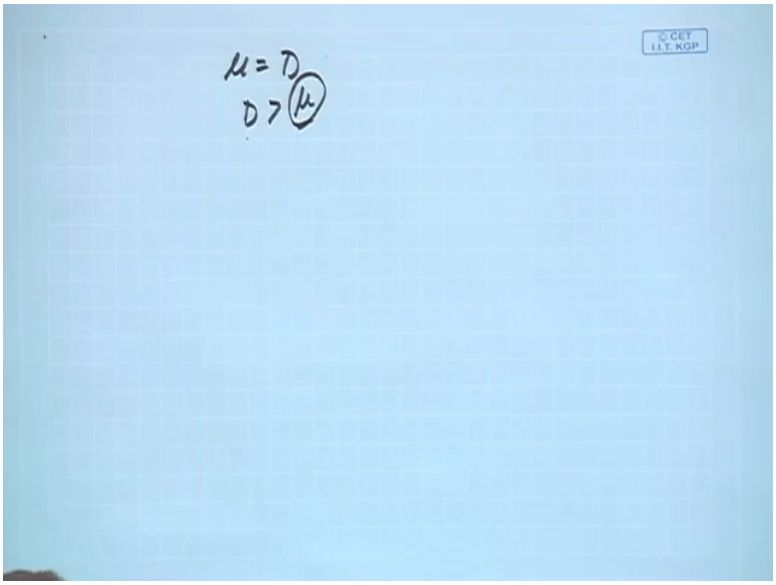

I have already shown you now here mew is a greater greater than D is greater than mu. So we can operate that your cell will not be wash out from the system. We can operate that that that that.

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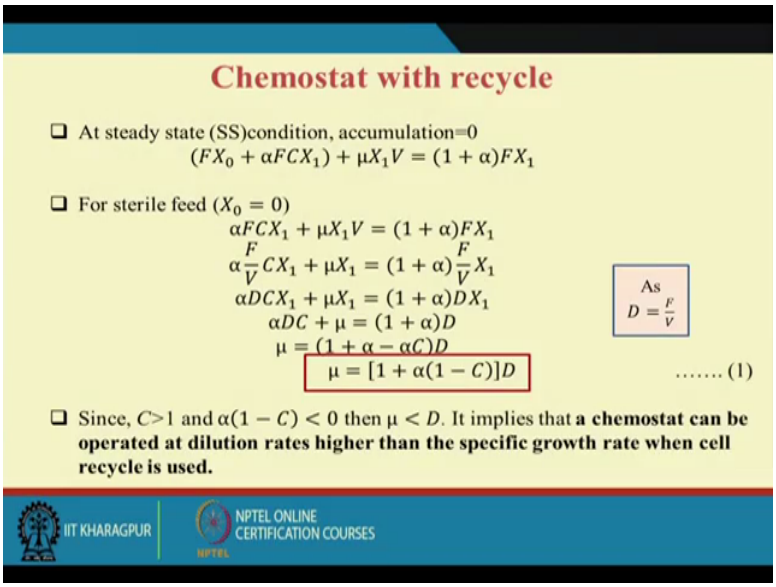

That is why we have written here chemostat that can be operated at the dilution rate higher than the specific growth rate when the cell recycling is used.

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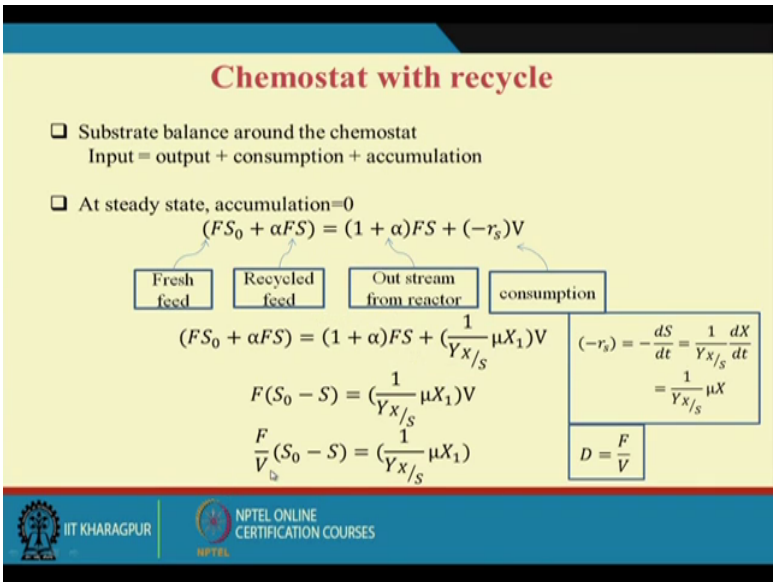

So you can this can be calculated in other way this is fresh feed FS0, this is recycle feed, this is out going and this is the subject consumption. This is on the basis of substrate balance we can also write the equation like this.

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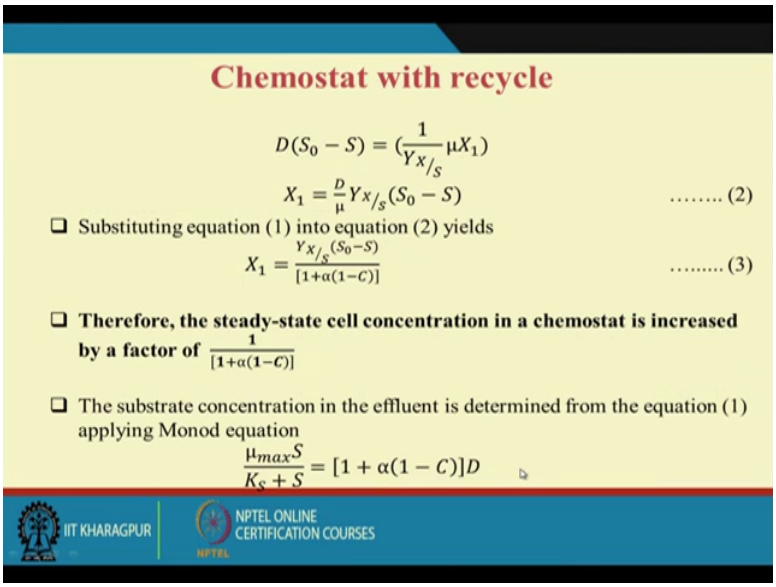

Now finally we come across this X1 equal to this value. The X1 equal to value can be taken therefore the steady state condition the chemostat increased by a factor. The cell mass factor is this one. The cell mass factor is by this. The substrate concentration of effluent is determined by the equation for monod equation can be expressed by this equation.

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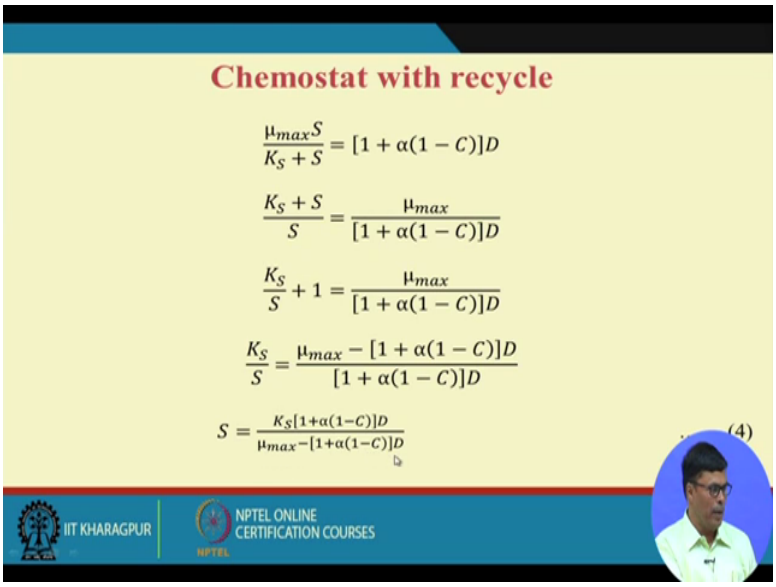

Now chemostat with recycling at further analyze and we find out the value of S like this.

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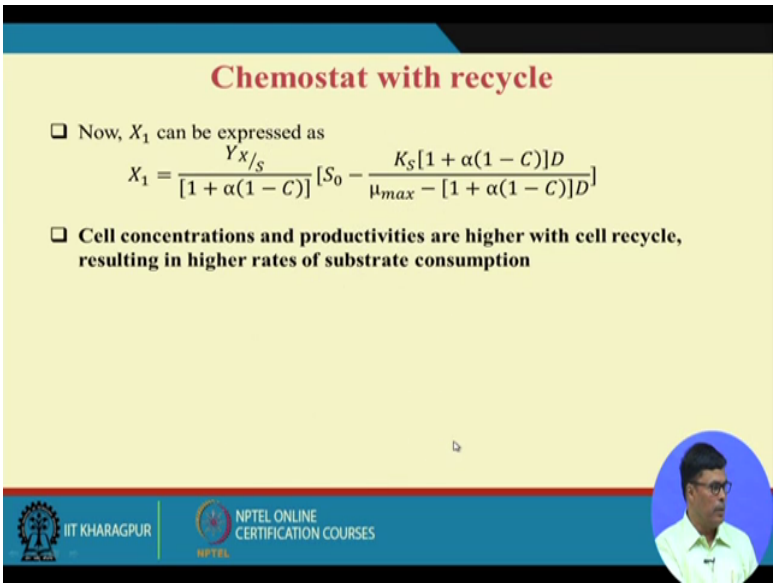

And then we can express the like this and the cell concentration and productivities are higher with the cell recycling result in the higher rate of substrate consumption.

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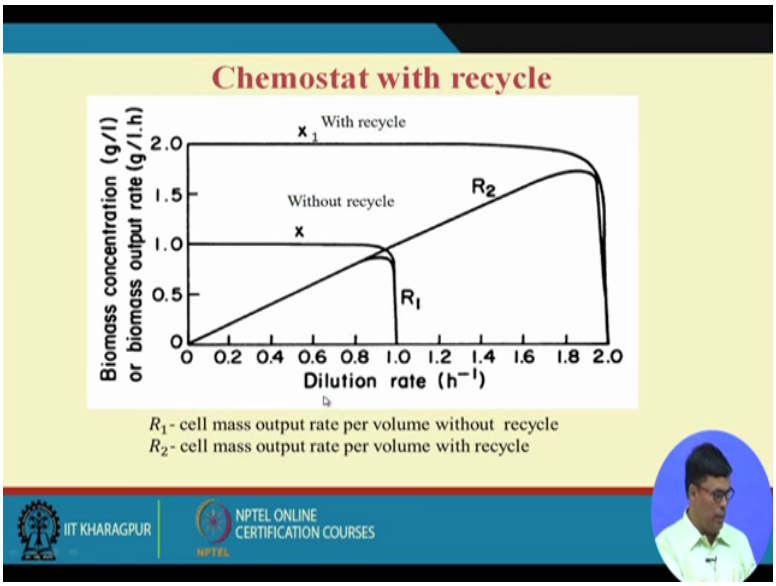

Now here that interesting thing is that

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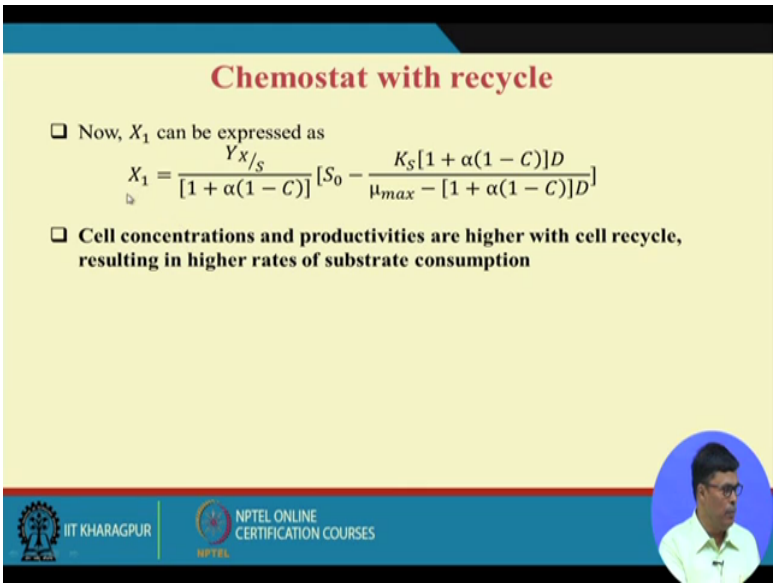

That here we find that X is increases.

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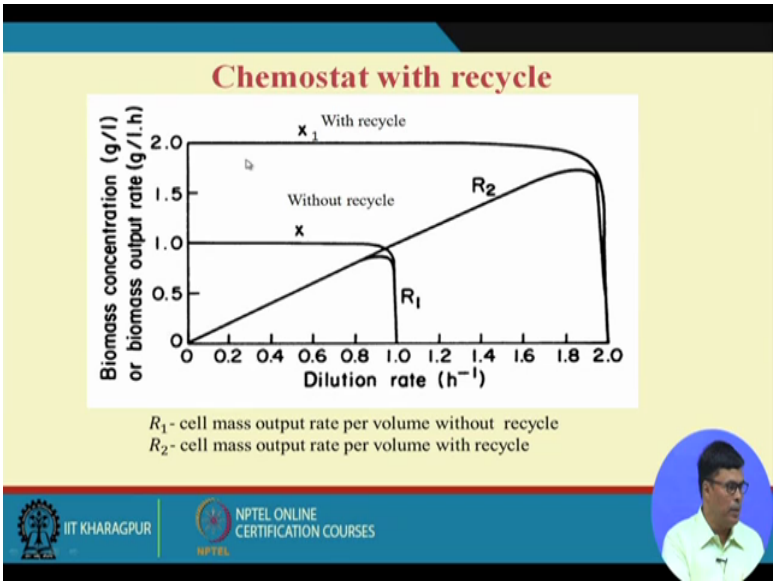

Now here that you see that in case of cell mass without recycling that X value that this is a dilution rate. Here we get a one might be the that C is approximately this is equal to cell wash out but when you do the recycling this is shifting to the two. That means you can operate it much higher dilution rate. Because that is the advantage of cell mass recycling that you know if you increase the dilution rate is increased if you if you do the recycling the dilution operating that can be increased a great extent.

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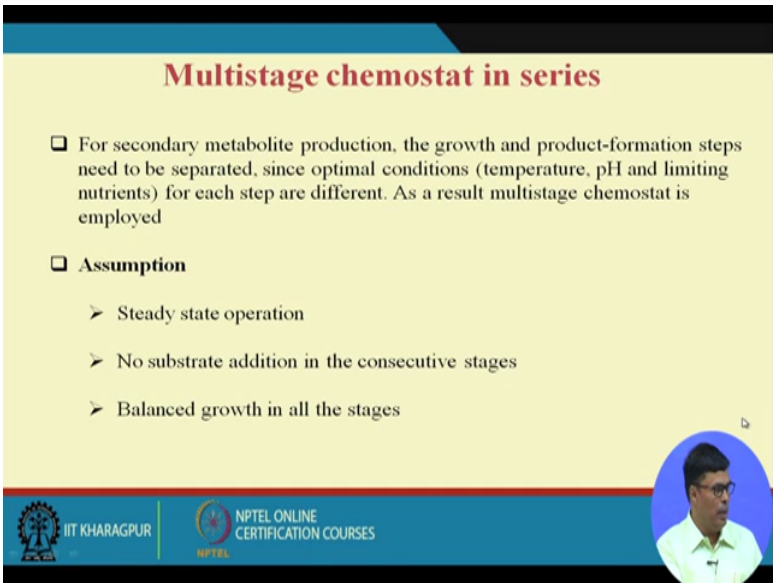

Now I want to point out that how to operate the multistage that chemistat in a series.

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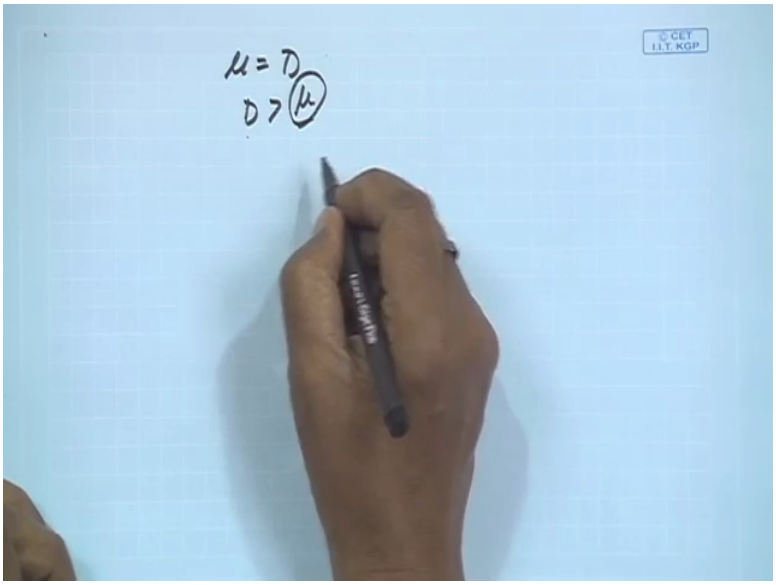
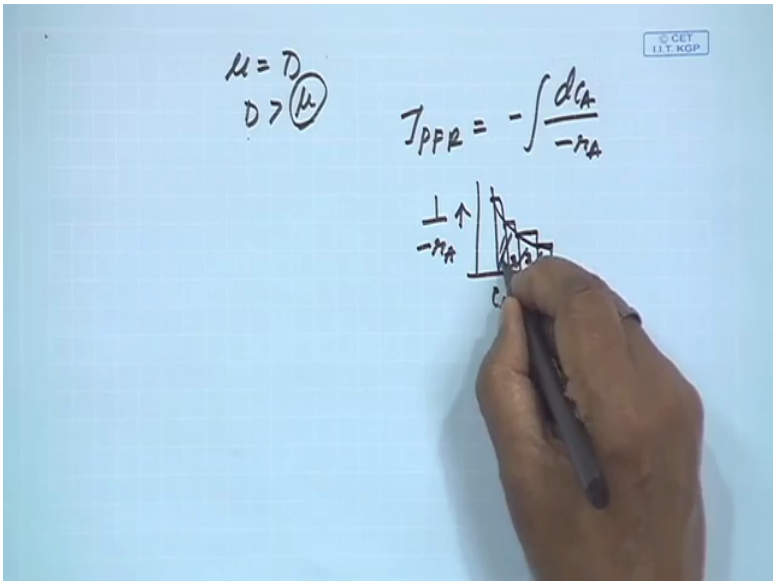
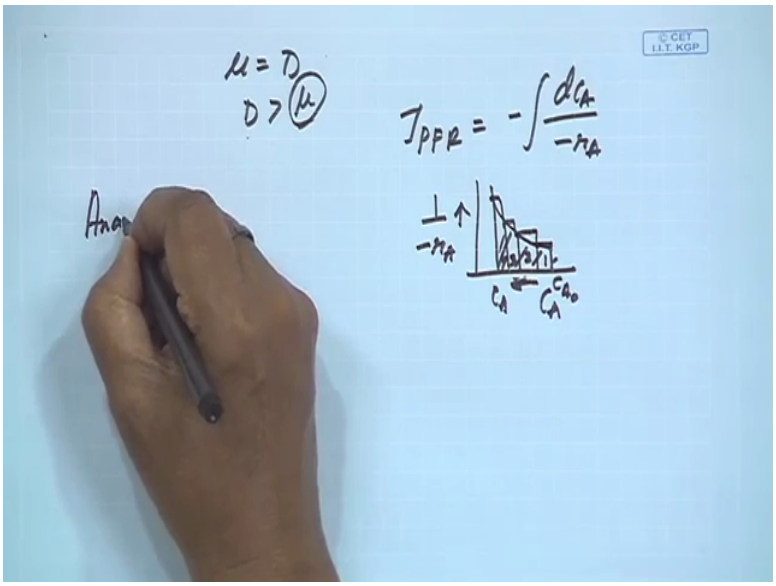
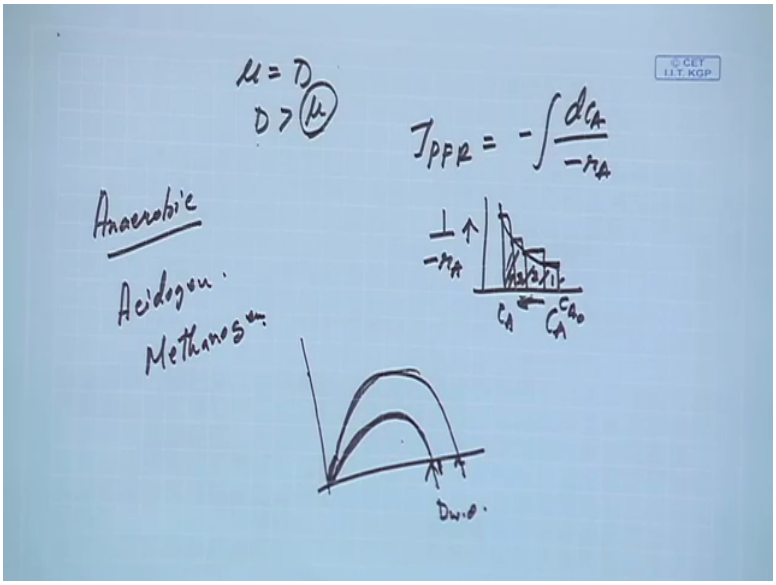




Multistage chemostat in series

- For secondary metabolite production, the growth and product-formation steps need to be separated, since optimal conditions (temperature, pH and limiting nutrients) for each step are different. As a result multistage chemostat is employed
- **Assumption**
  - Steady state operation
  - No substrate addition in the consecutive stages
  - Balanced growth in all the stages





Because what you call cascade type of fermenter. Cascade because I you can you can remember that in case of reaction kinematics we have come across this plug flow reactor. In case of plug flow reactor we have come across this equation. This minus DCA equal to minus RA. Now here I try to point out that minus RE if you plot by CA.

And if you have this this kind of thing and this is the this is the as the CA value decreases this is CA 0 to CA 1 by RA increases that means RA decreases. That is actually take place in case of product innovation because because as the substrate concentration decreases the innovation occur that means rate of reaction decreases and at this situation plug flow reactor is mandatory.

Now we observe this plug flow reactor is very difficult to operate because plug flow reactor there should not be any kind of back mixing. Now if you have been in place of that if you have multiple CSTR in series then that will be equivalent to plug flow reactor. 1 2 3 4 so this is exactly we we try to find out for secondary metabolite production that the growth and product formation steps to be separated optimal stage.

And multistage of the state process that we can not only that we can we can have because I can tell you this very interesting that in the anaerobic dilution process we have to type of culture one is called as acidogou and another is methanogens is going as compared to be the reasons so if you if you if this is available if you want to separate the acidegens from the methanogens you can do it very easily.

Because you have you have this picture is like this one is this and that is this so this is this is for your methanogens and this is what acidegens. So if you here this is D wash out. For this is methanogens and this is D wash out for methanogens and acidegens. So if you use the dilution rate higher then this methanogens will be totally wash out from the mixed culture so.

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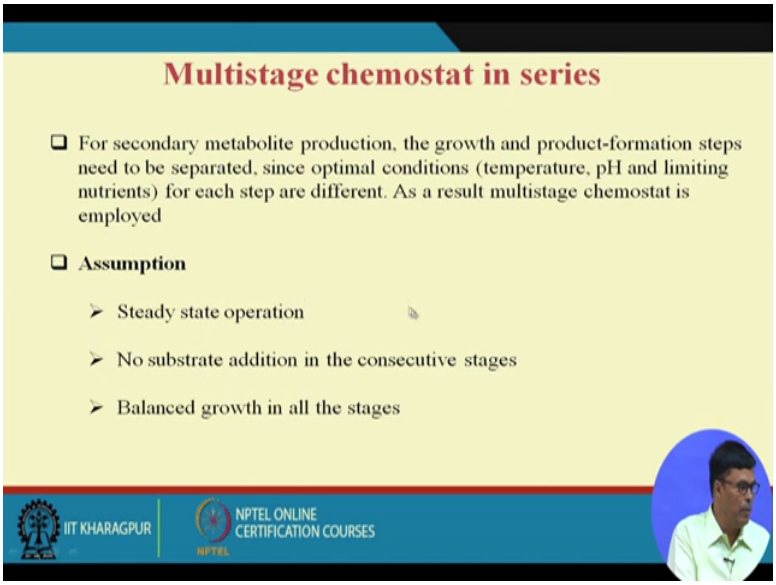

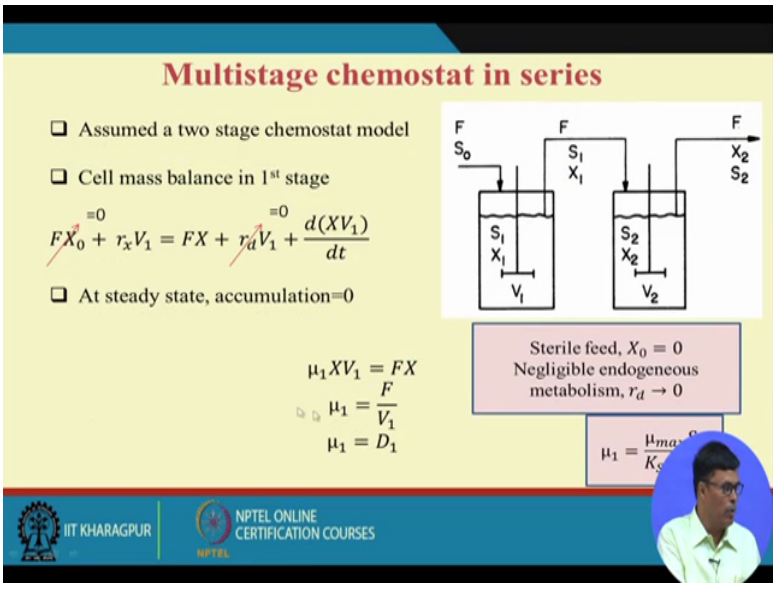

This is another advantage of this the different with the help of dilution at we can do this. Now in case of multistage and this is usually assume the steady state operation. No substrate addition for change of phase unbalanced growth occurs in this is like this this is substrate is coming again whatever substrate ()((28:29)) we put it in the second reacted.

So then more substituted but for the first reacted we don't have analysis is not problem. Because we assume it in the serial field we have already done we have to easily find out the value of S1 and X1 in this reactor.

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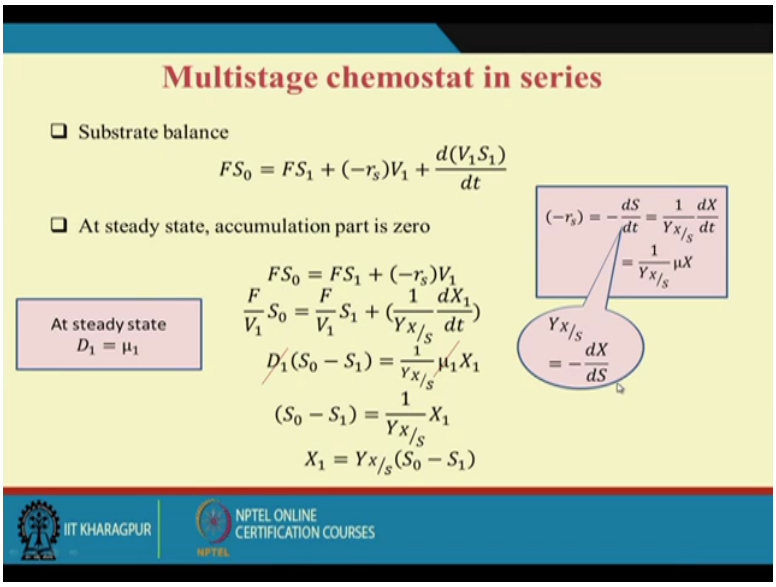

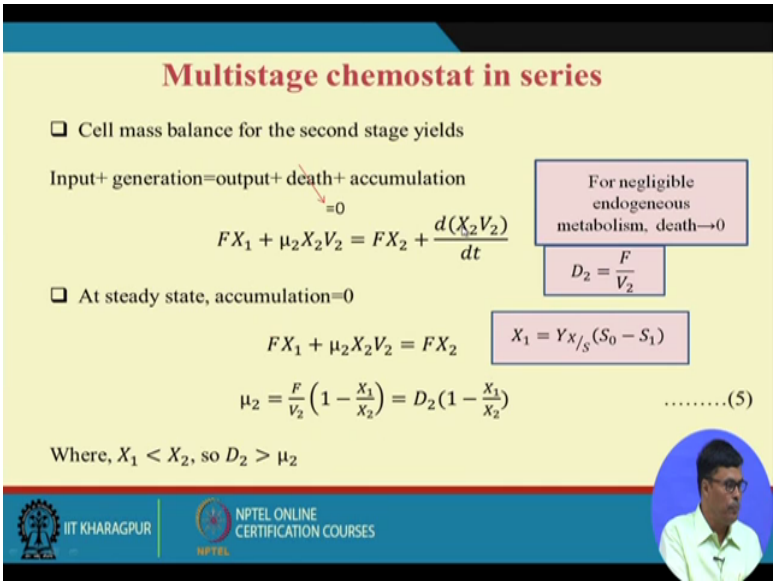

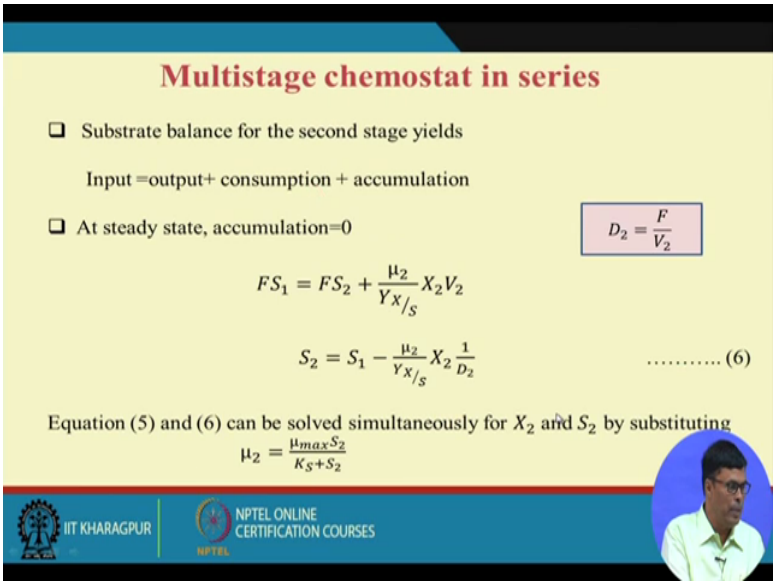

Now when you go to the second reactor, we we shall have to do the material analysis. We shall have to do the substrate balance and we shall have to find out what is the what is X1 and X2 value that you have. We can calculate S2 and X2 value in the second reactor. We can calculate separately then you you want to go to the third reactor.

And you have to do the similar type of analysis like this you can you can do the cascade type of analysis of the different reactors find out the different cell mass concentration and different type of reactor.

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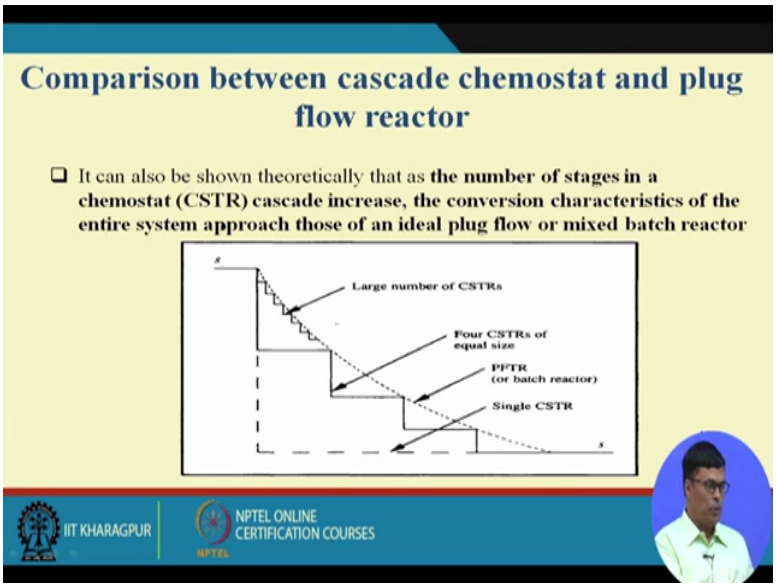

So let me stop here today because we shall we shall continue this lectures and then the next class I will discuss some problem also so that whatever things I discuss this will be little bit clear to you. Now in this lecture I tried to point out that the major drawback of chemostat process is that cell mass that is wash out and this can be overcome by the recycling.

And by through recycling is it possible not only to operate at high dilution rate but at the higher dilution rate we increase the cell mass concentration to a great extent. The major concept of of the cell D wash out I try to explain that 1 by D is considered the higher retention time if we have hydraulic retention time less than that cell the cell division time required for the generation time.

Then say you are not ()((30:31)) the cell to grow that is why the cell wash out take place on how we can explain in other way if a Cell mass that is going out of the reactor is more as compared to cell mass that is growing in the system. If you allow the system to continue for longer period time we always meet the situation of D wash out. So this we can we can we easily separate by using the recycling of the cell.

And also also we discussed in the last previous classes immobilization of the whole cell. The immobilization of the whole cell we can hold a cell in the solid metric so that no wash out of the cell will take place. So we discuss I think in the coming lectures we try to try to discuss some compression of the chemostat and plug flow reactor then I shall discuss about this some

problem so that your how this can be applied for solving some kind of fermentation in the problem of the fermentation industry. Thank you very much!