Course on Industrial Biotechnology
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Lecture 18 Life Cycle Of The Microbial Cell, Microbial Growth Kinetic, Product Formation And Substrate Degration (Contd.)

Welcome back to my course industrial biotechnology. So in the last lecture we try to cover the life cycle of the microbial cells, microbial growth, kinetics product formation and substrate degradation.

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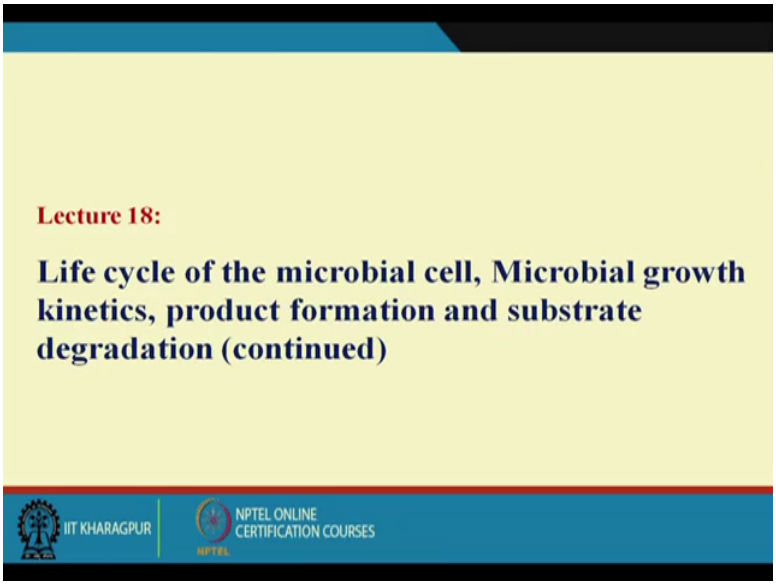

So will continue that and now let us let me let me somewhere I thought I covered try to cover in the last class that we we discuss about the life cycle of the cells and we also discuss what is the important of the life cycle in the microbial m cultivation system or in a in the fermentation process and we told you that when the life cycle we have lack phase, we have Lock Phase, we have stationary phase and we have dead phase.

The every phase has some significant lack phase till that limitation period. Lock Phase is called the active growth phase and stationary phases of starvation phase. Now one thing we should have to remember that in the fermentation industry we have two things simultaneously.

We have one is inoculum preparation and that is a production for winter. In inoculum preparation we try to prepare the culture and the volume of the culture usually 5 to 10% of the production for winters. So suppose I work with citric acid industry and the two

formantant we have the capacity of 200 cubic metre and another formatted was 225 cubic metre.

Now if you considered 5 to 10% is around 10 cubic metre to 20 cubic metre. So this much is huge volume of culture can be produced in the lab. So we set up to produce in the plant. Now when when you prepared this culture will shall have to see that that culture whatever inoculate from the lab it should be in active phase. And that is why the study of life cycle is very important.

Because the if we life cycle you'll find that one is the lock phase and usually the cultivation is done in between the lock phase to lack phase and then I told you also that most of the sum of the most of the fermentation industry produce secondary metabolism. I have given the example of penicillin in different antibiotics industry.

Production industry like penicillin streptomycin they they the secondary metabolites and they produce in the stationary phase since the stationary is this starvation phase we try to extend the stationary phase so that we can get maximum amount of product. After that we try to discuss the the role of batch process and the chemostat batch process usually have mostly used for determining the life cycle of the self.

The disadvantage of this process that we can not hold any phase of growth for infinite period of time. So that is the major drawback of the batch process suppose if we want to produce the Baker's cyst that the cell mass production maximum take place in the Lock Phase, so since we cannot hold the Lock Phase for longer period of time then which really that we can get the higher amount of sale mass productivity.

But in case of chemostat process chemostat is nothing but chemistry in the biological system and the chemostat process I showed you that meu equal to d meu is the specific growth rate, d is the dilution rate and so dilution rate is basically is equal to ()((4:12)) the volume of the flood divide by volume of the reactors.

Volume of the reactor working volume of the format that is remaining constant, if you change the product you can change that dilution rate. So at different dilution rate you have different steady state condition. So single so we we try to develop the correlation between the status still still mass concentration, steady state substrate concentration with respect to the dilution

rate also we try to correlate how the rate of still mass productivity correlate with the dilution rate.

Then we determine that we will find out the equation for d-max d-max Transport the maximum dilution rate where we get the maximum rate of cell mass production. Also we come across another point d wash out. D wash out is the dilution rate when there is no cell present in the reactor and try try try to explain the physical significance of that that every cell has generation time.

And one by the d user hydraulic retention time if you don't allow the cell to resize in the reacted for longer period of time the before it multiplies we could take how do you not you not get cell in the reacted after sometime. So this is this the disadvantage of the CST process and disadvantage disadvantage can be overcome by using the CST address elmas recycling that we will try to discuss how the how this system can be analysed.

And and and and then we will also try to give you better tell you how the plug flow reactor can be used. Now major problem with the plug flow reactor is the is the there should not be any kind of back mixing in the system. So this back flow reactor can be used for the system where we have the product innovation water and this can be replaced by using the multiple CST here.

Now today will go ahead with this and we will discuss some information that is left with us with this with this (())(6:31) with cell mass recycling. Then I shall discuss the pot model and alluring pirate model because alluring pirate model deals with the rate of product formation in the fermentation industries. How it is correlated to the cell mass concentration and what model is a little sis the maintenance of the cells and this will be followed by some numerical problems. Let me start with that.

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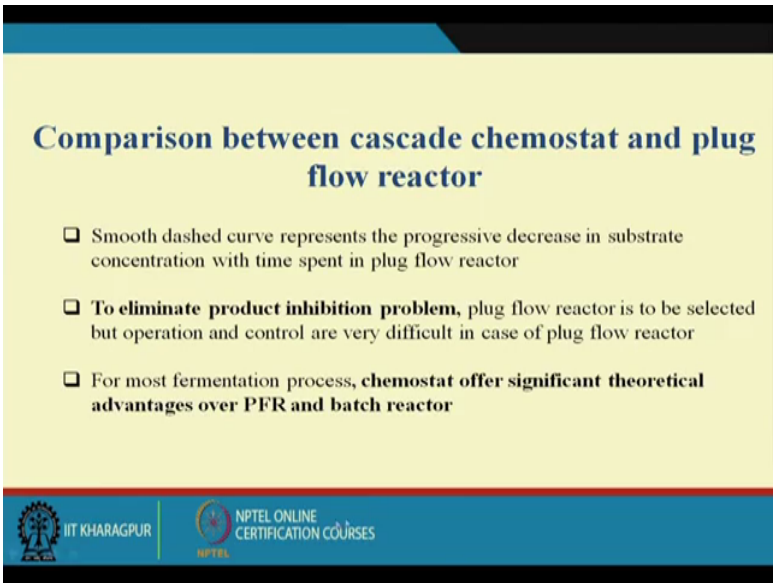

This is the comparison between the cascade chemostat and the plug flow reactor.

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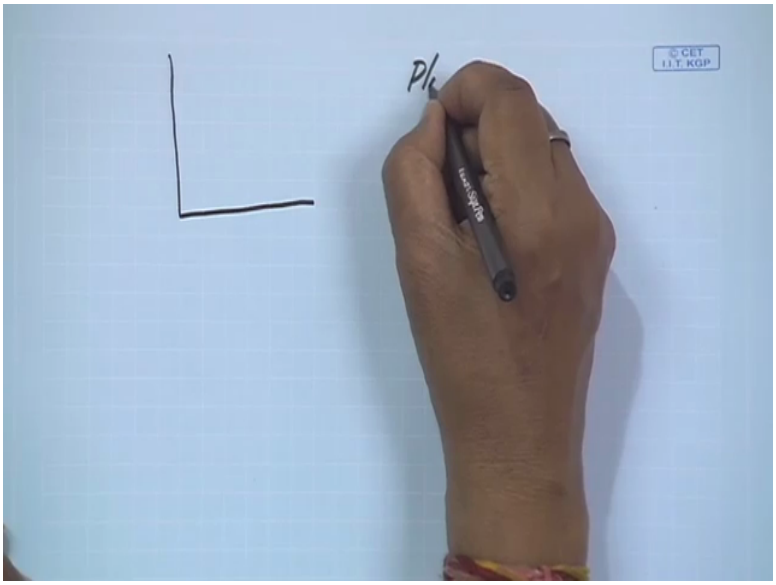

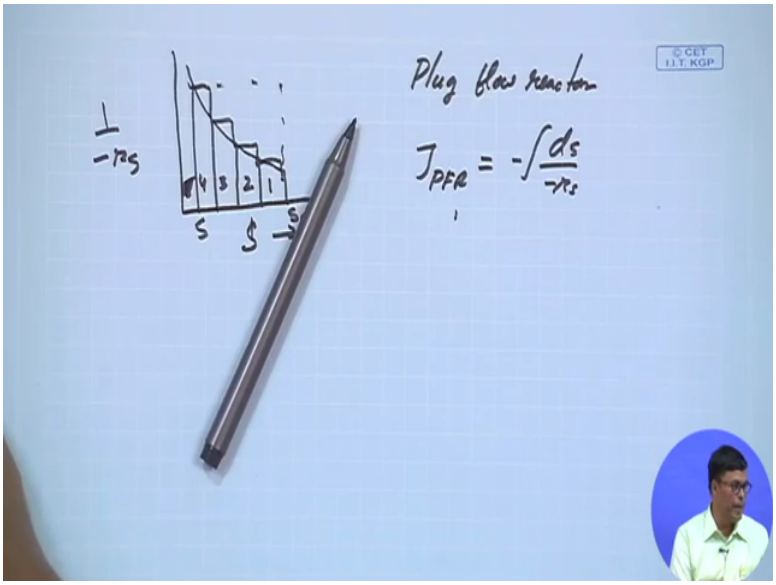

Again let me explain that in case of plug flow reactor tow plug flow reactor is equal to minus ds minus rs. So if you if you plot s this way minus1 by rs if you have this kind of this is this is a zero and this is s. Now I told you as s increased decreases that you know one by rs increases that rs will increases that is example of the product innovation.

So in case of CST that area under this called will be this which is much higher than area under for this plug flow reactor. So here the plug flow reactor is usually preferred. Now say that plug flow reactor is very difficult to operate. It can be replaced by using the multiple CST. What do you call the cascade formantate. This is so this is first reactor, second reactor, third reactor, fourth reactor, fifth reactor. So the five reactors sorry four reactors in series will be equal into this this last one reactor.

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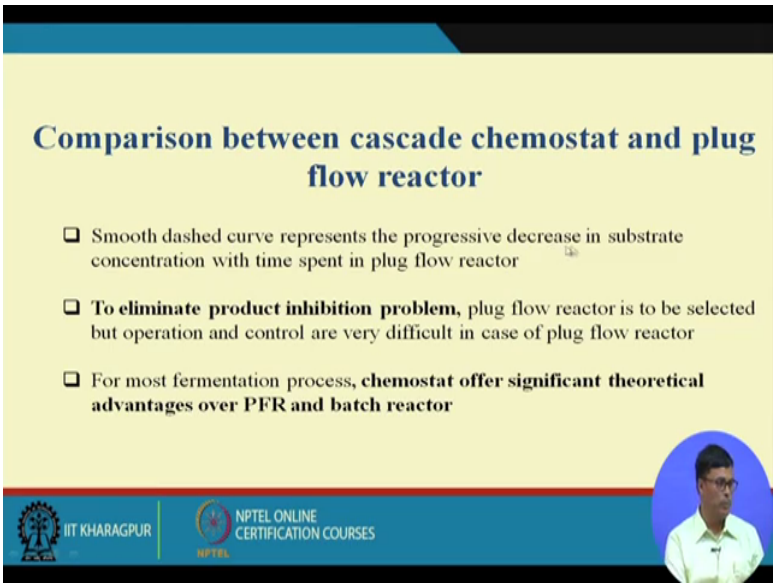

So smooth dashed curve represent the progressive decrease in substrate concentration with time spent in the plug flow reactor. To eliminate the product innovation problem plug flow reactor is to be selected but operation and control are very difficult in case of plug flow reactor.

Almost fermentation process chemostat offer significant theoretical advantage over the plug flow reactor and the batch reactor. That is that is that is why the plug flow reactor is replaced by the cascade reactor. Cascade reactor cascade formantant is usually that cstr in series then that will be equivalent to plug flow reactor. Now I was discussing about CSTR with cell mass recycling.

This is this is the typical example of activated sludge process when it is largely applicable (()) (9:32) in the different chemical and bio-chemical industries. In fluent is coming then after this is the format after that he passes through the separator the supernatant you take out and the city of a part of the citizens you do cycle back and (())(9:48) much you take out from reactor. Now here we have now we need kind of recycling one thing I tried to I I forgot to mention the purpose of recycling is to increase the retention time off the cells. But because we have we have come across.

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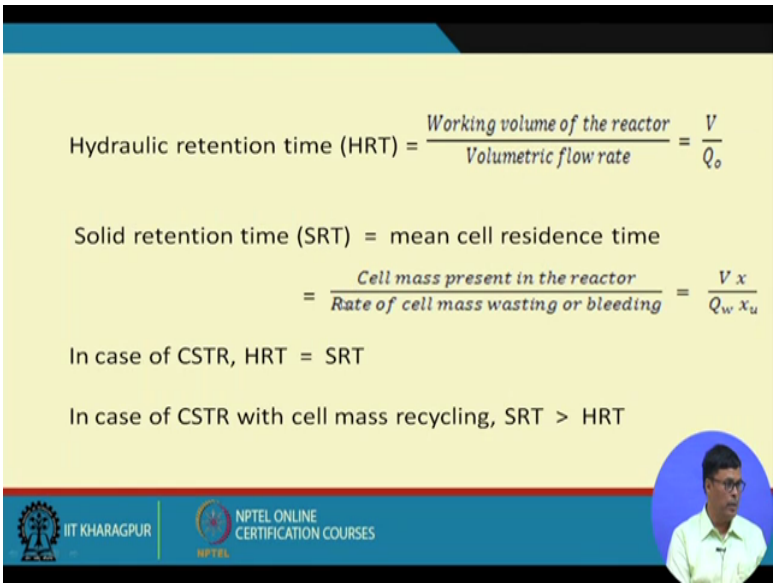

You can you can see that hydraulic retention time is what that volume of the reactor divide by volumetric flow rate. So this is the this is the hydraulic retention time.

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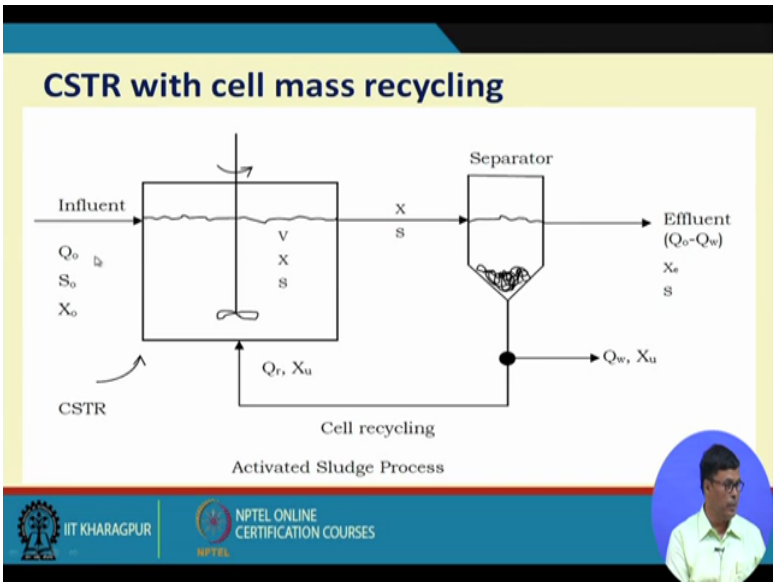

Now now this is like this this is the flow rate Q0 is a flow rate. And v v is the volume of the reactor.

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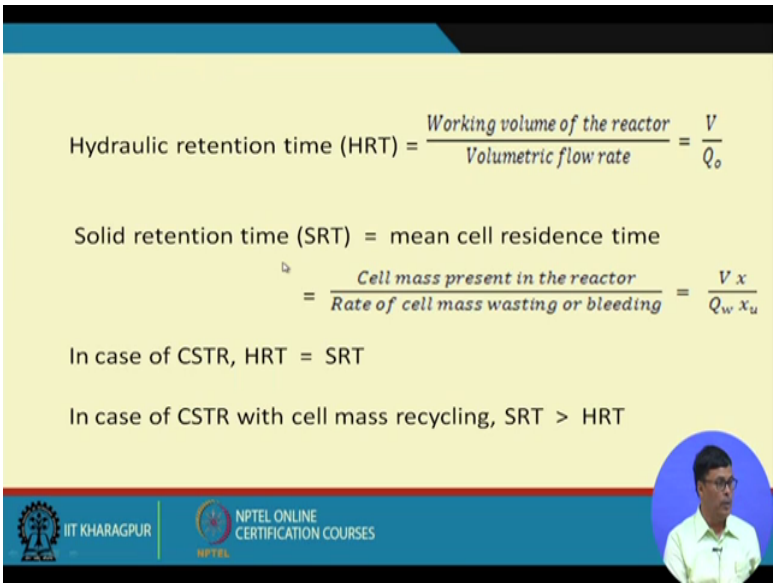

Now here v divided by this is volume per unit time so it will be time. So this is hydraulic retension time. So what is the solid retention time?

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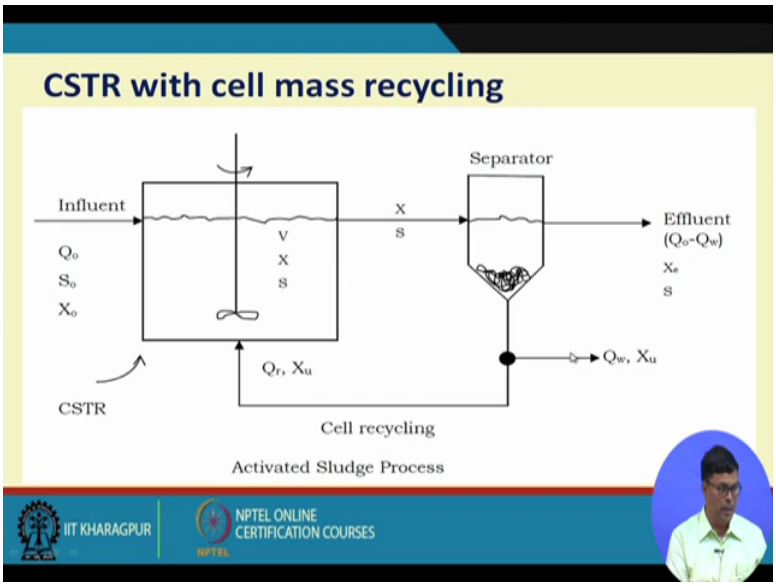

Solid retention time here the same as that is going out of the system is not coming back to the system. The actual cell mass loss from the system as shown if this x is negligible as compared to this. Then actually what is this this is the rate of flow of (())(11:01) that is going out of the reactor and this the concentration of salt. So you know that cute Qw into xu what is the unit of Qw? Qw is the volume per unit time. Am I right?

And what is the unit of xu concentration is the mass per unit volume. So volume volume will cancel this is mass per unit time that is what do you call this? This is called cell mass wasting. Cell mass wasting wasting wasting means you are taking out from the system. So this is like this.

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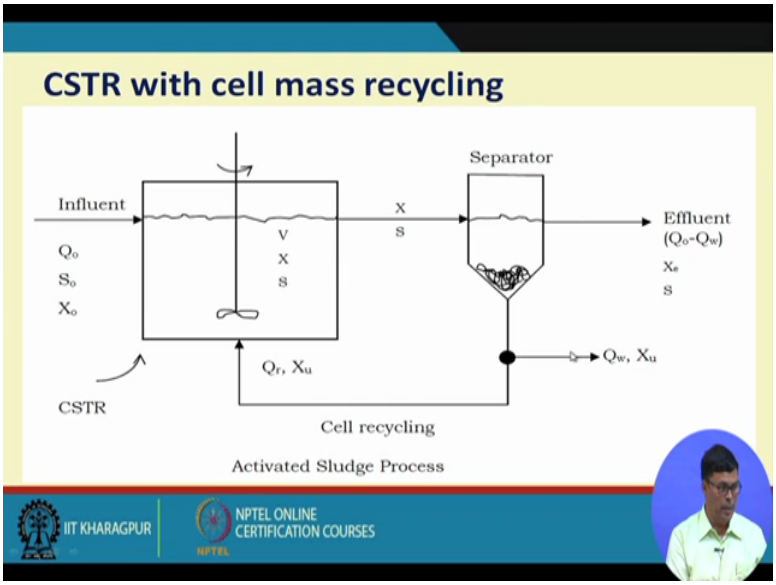

So now how much cell mass is present here because we are shown here most of the reaction take place only in the reactor. No reaction takes place in the pipeline or in the separator. We assume that now if the number of sale amount of cells within the reactant is what the V into X. V is the water volume of reactor, X is the cell mass concentration.

This is a total amount of cell mass present in the reaction and how much you are taking out for you need time then Qw into Xu.

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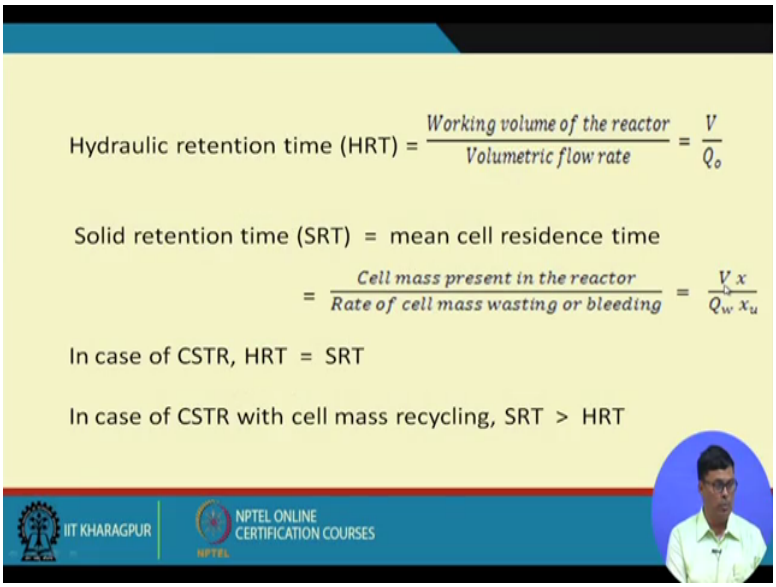

So that is why the solid retention time is equal to what V into X into divide by Qw into Xu. So the main purpose of recycling is to increase the solid retention time. We want to because if you if you don't recycle then that retention time of the cells and it is in time of the liquid will be same.

The hydraulic retention time is required to the solid retention time, but as soon as you recycle back the cell to the reactor the solid retention time increase. The main purpose of recycling of the cell you want to increase the solid retension time.

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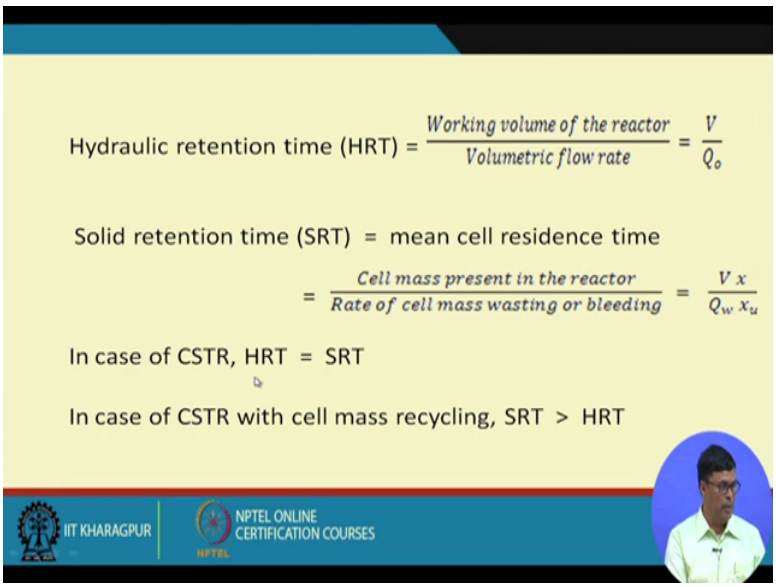

So that is exactly have written that in case of cstr the hydraulic retention time is equal to Solid retention time. But in case of cstr cell mass recycling solid retention time is much higher than that of hydraulic retention time.

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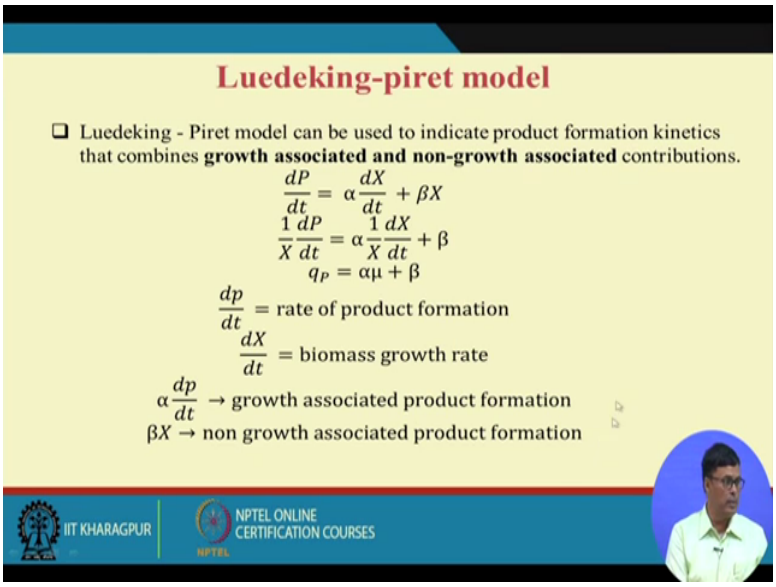

Now let me discuss about luedeking-piret model. This is very important. The luedeking-piret model can be used to indicate the product formation Kinetics that combines the growth associated and not grow the associated contribution. Now let me explain that explain in details what do you mean by that growth associated and what you mean by non growth associated product.

Before that let me let me explain that luedeking-piret model. Luedeking-piret model is the dp by dt. Dp by dt is the rate of product formation equal to Alpha. Alpha is the growth associated Coefficient DX by DT and beta into x. Beta is done on growth associated position. Now if you divide by 1 by X why I told you before as early as soon as you divide dp by dt by 1 by X we call it specific product formation rate.

Now this is dx by dt whole divided by 1 by X, we call it specific growth rate. So this is the specific product formation rate will be Alpha into meu into beta. The beta is constant always constant. So so we can we can easily plot.

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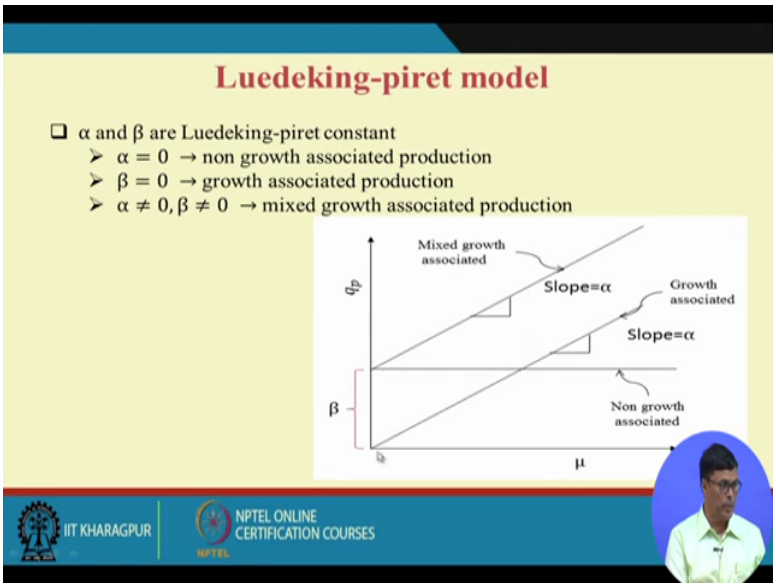

We can we can plot up into this is a straight line equation and if u it passes through the origin it passing through the origin then beta value should be equal to zero.

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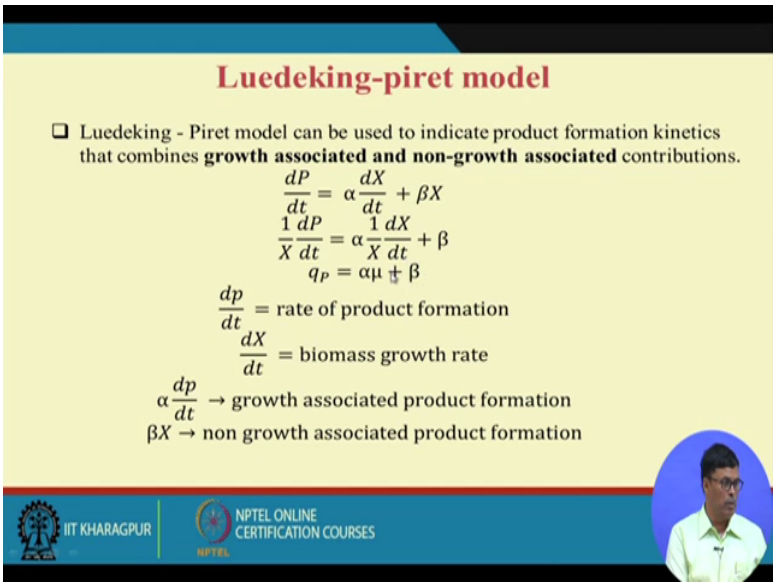

I need the beta value is equal to zero we call it growth associated products. I will explain you how the growth associated why we call it growth associated products.

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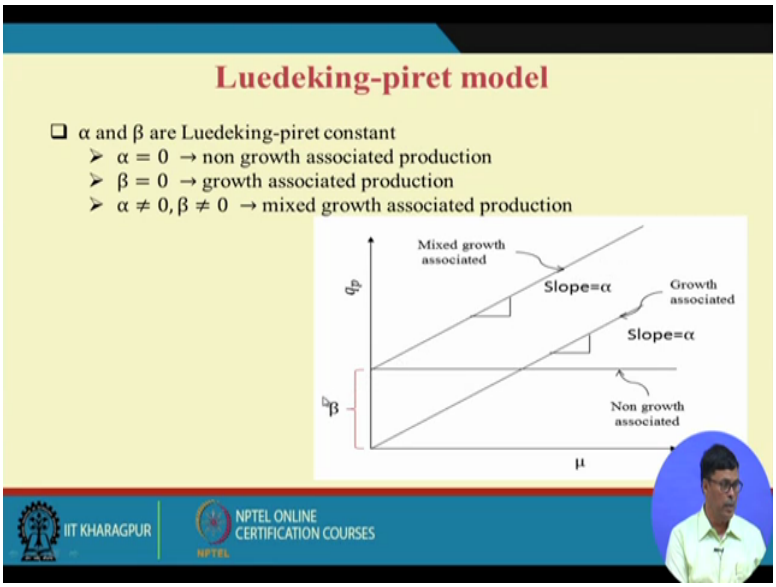

Now in case you your plot is like this. This is your interest will be beta and slope will be alpha in that case we call it to make growth associated product. Now in but we have non growth associated product that in case of secondary metabolite formation that we have not go the associated product in that case what will happen?

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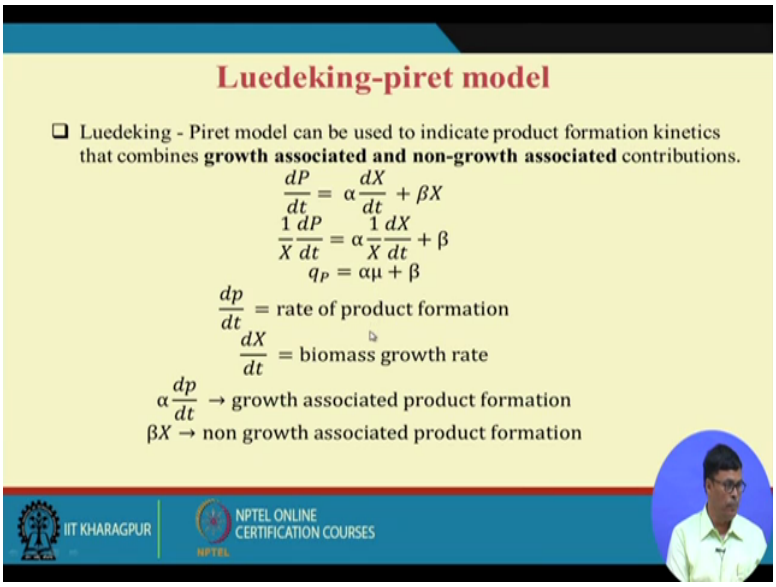

That that Alpha will be equal to zero. So then dp by dt actually equal to beta into X. Because it depends on the same mass concentration and it will be a mole is a straight line that we have. So far so far this is if a plot with X, it will change with respect to then you can easily

find out the value of Beta. Now let me explain you how you can define the growth associated products and non growth associated product.

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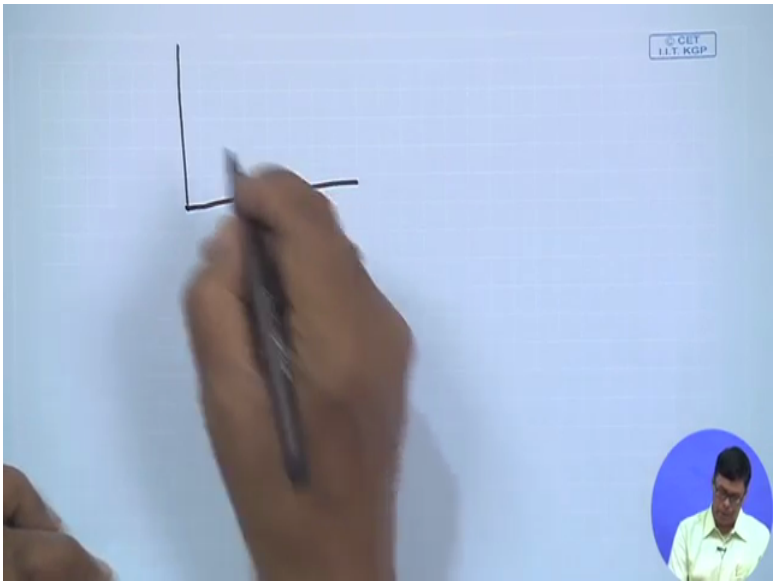

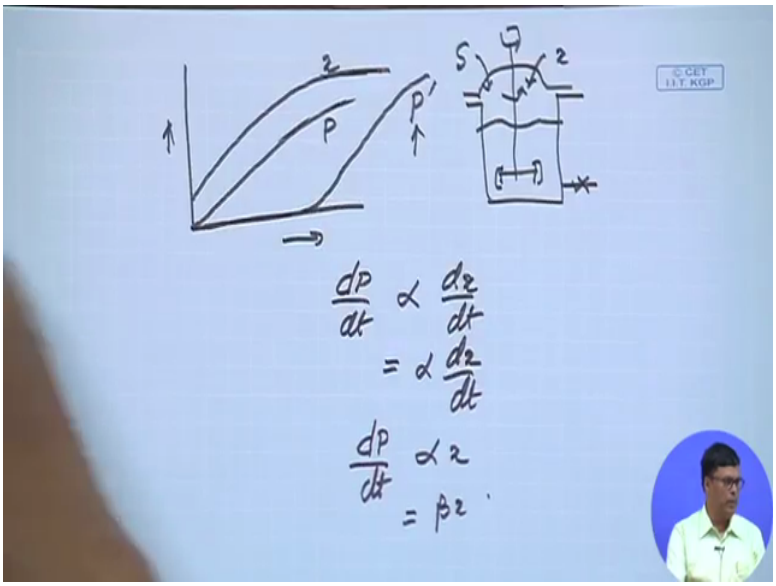

Suppose a fermentation process you this is let us consider this is a batch fermentation process. Here you take the substrate on media and then you put the cell mass here and time to time you monitor that this mass concentration will increase like this with respect to time. Now if your product formation also increase like this.

Now you see this is product and this is cell that the product is other than the cell then it if it also changes like this in that case I can write dp by DT is proportional to dr by dt. That is exactly what you for what is called that product growth associated product and this is equal to

alpha into DX by dt. Now in case of non growth associated product what is happening? When your organisms come almost in the stationary phase then your product formation start.

This is like this. This is P dash. This is the product was this is an example of non growth associated product because rate of product formation obviously proportional to the cell mass concentration. But this is this this is occur when sell that in the the stationary phase. So this is equal to beta into x. And this is and here the example of the non growth associated product is different antibiotics fermentation process.

Citric acid fermentation process and growth associated product we have the alcohol fermentation process with another vehicle we can we convert the glucose into alcohol. So this is an example of growth associated product.

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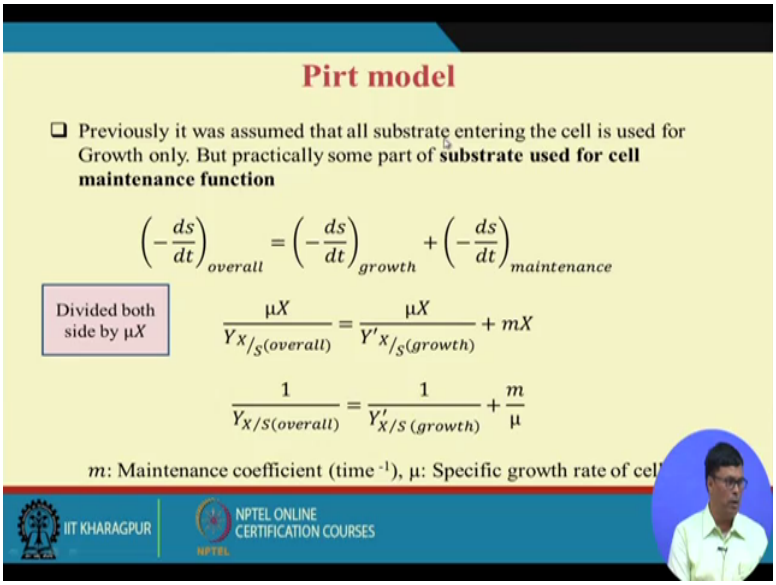

Next let me discuss about the pirt model. Pirt model previously it was assumed that all substrate entering into the cell is used for the growth only. But practically some part of the substrate used for the cell has the maintenance function that means whenever we use some kind of substrate a part of the substrate goes for the cell mass formation. A part of the substrate goes for the maintenance of the cell.

Now what you mean by maintenance of the cells? Maintenance of the cells means cells has some motility because in the liquid cell moves from one place to other and this motility of the cell require some energy and so this is this is one how the maintenance energy use not only that purpose for the cell captured because they are due to some other reason due to shear

force and other things the cell may after you have to repair the cell you request some energy. So all these different different protein formation also we required some kind of energy that will be considered as a maintenance energy.

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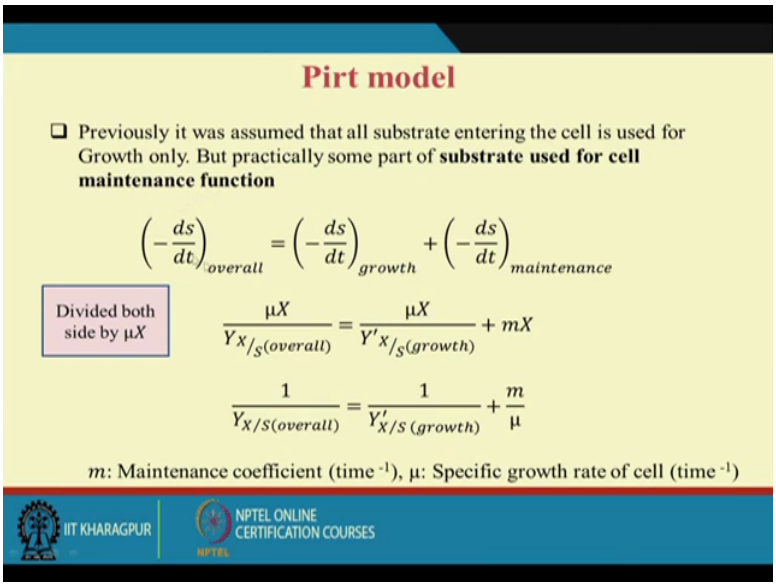

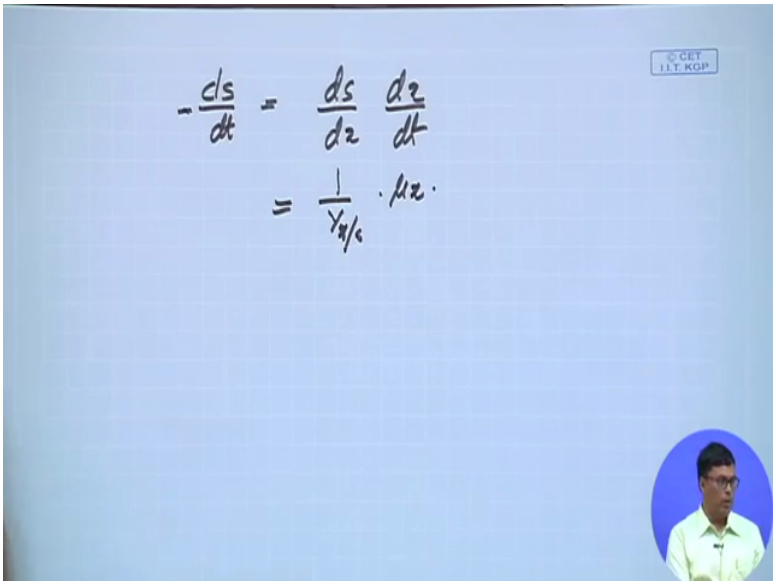

Now now if the pirt equation is like this. The rate of substrate degradation is equal to that of state that is used for the growth. And rate of substrate that is used for the maintenance of the cells. Now I I already explained you now if you if you write ds by dt ds by dt minus ds by dt what I can write?

This is equal to ds by dx and this is dx by dt am I right? Now if you if you this ds by dt is nothing but by x, this is called nill Coefficient and this dx by dt is equal to meu x. That we have we already did it in all the class.

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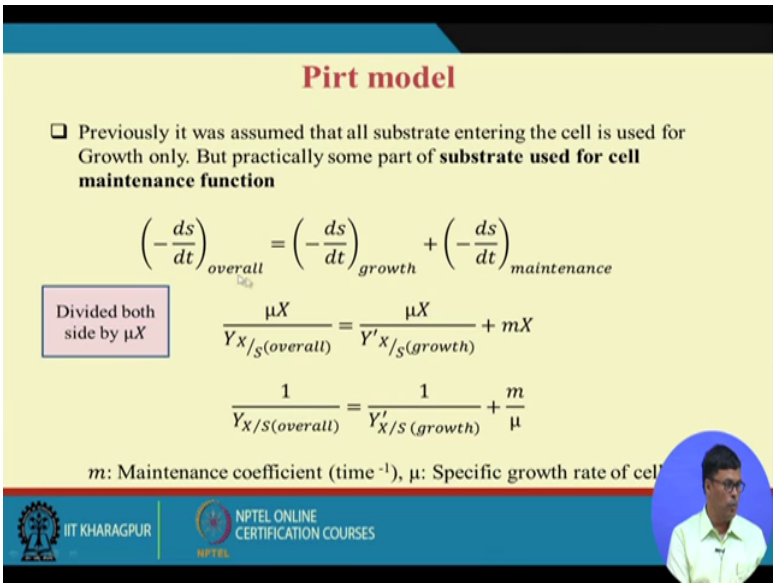

So we can substitute this minus ds by dt overall we can replace by meu into x y x by s overall meu into x y dash x by s. Now here I want to point out like this here the rate of substrate they evolved in the that consumed overall substrate consumption is equal to the substrate consume for the growth of the cell and substrate consume for the maintenance of the cells.

Now but this we can write like this for the growth of the cell this overall growth of the (()) (20:27) we can replace like this meu x by y dash overall and this is growth we can write meu x y dash x by s. Now why does x by s is considered as true yield Coefficient?

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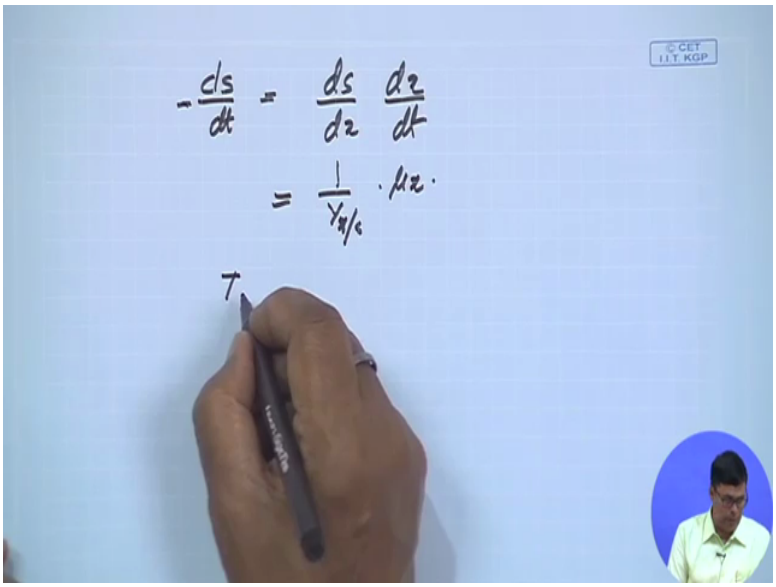

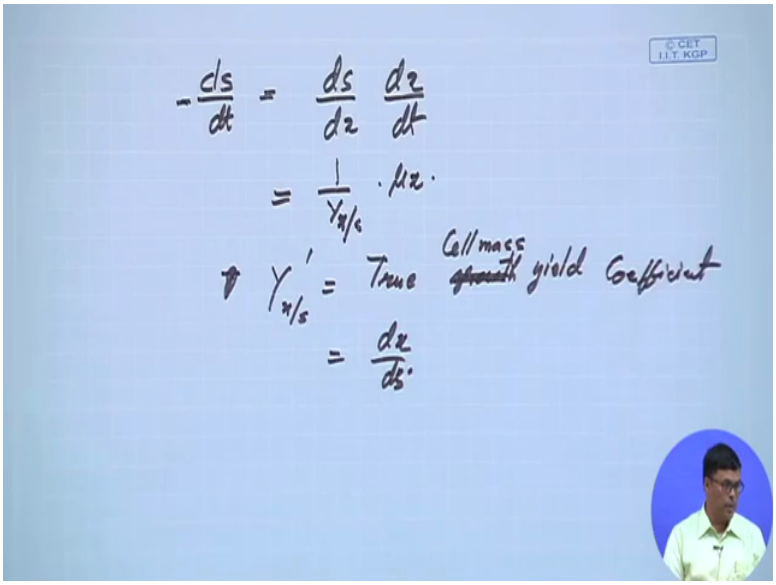

This is y x by s dash is called true growth yield. Growth yield all we can write in the Silver Shield. That means the this is equal to also dx by ds, same as overall that you know overall yield coefficient but here these dx by ds means the (())(21:22) of substrate how much cel mass produce when all the substrate is used for the growth of the cell only, no it is not going for the maintenance of the cell that is called the true yield coefficient. This is Coefficient. Same this we can write this.

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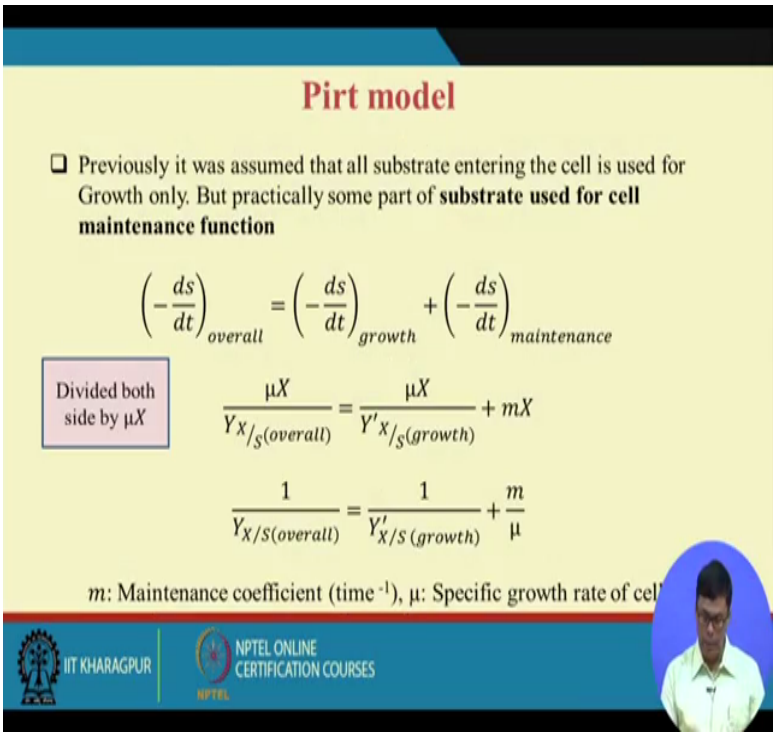

Now another thing I want to tell you that rate of substrate used for the maintenance of the cell this is proportional to what this is ds by dt.

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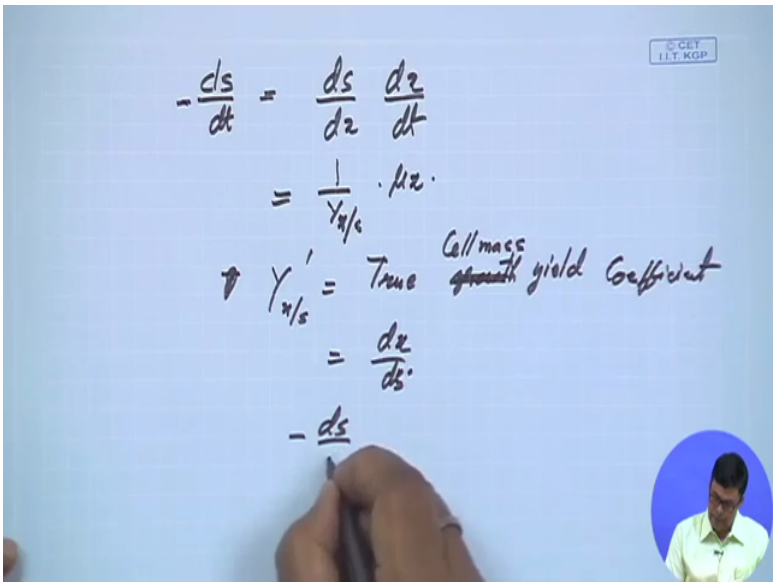

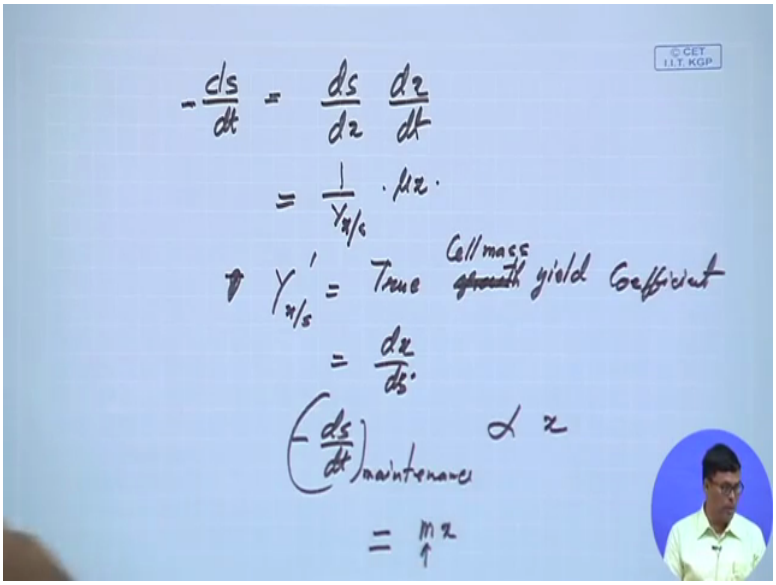

If you write this is used for maintenance. Now this is proportional to the cell mass concentration. More cell mass concentration, more will be the maintenance. Less cell mass coefficient, less will be the maintenance. That is when you equal this this is m into x. M is that what you call maintenance Coefficient.

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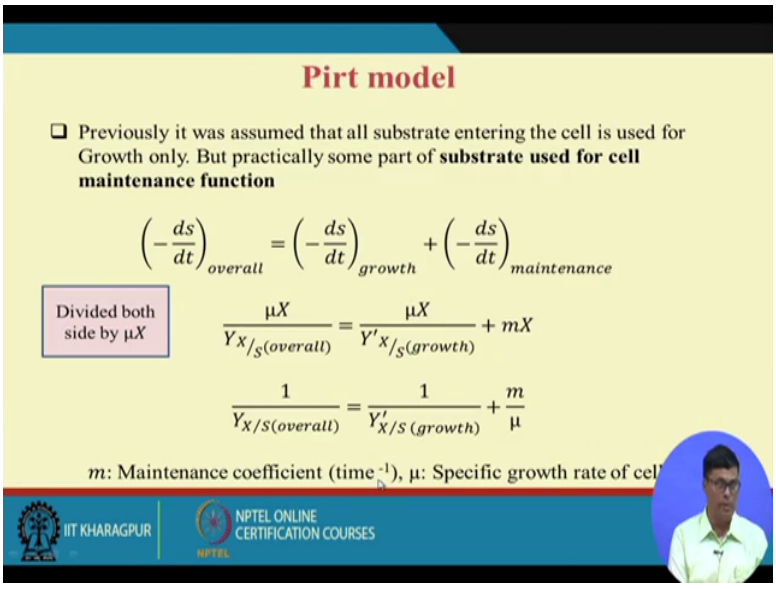

This is call maintenance condition and unit is time inverse. This is call maintenance coefficient and unit is time inverse. So here interesting that if you plot now if we plot 1 1 by y x by s overall.

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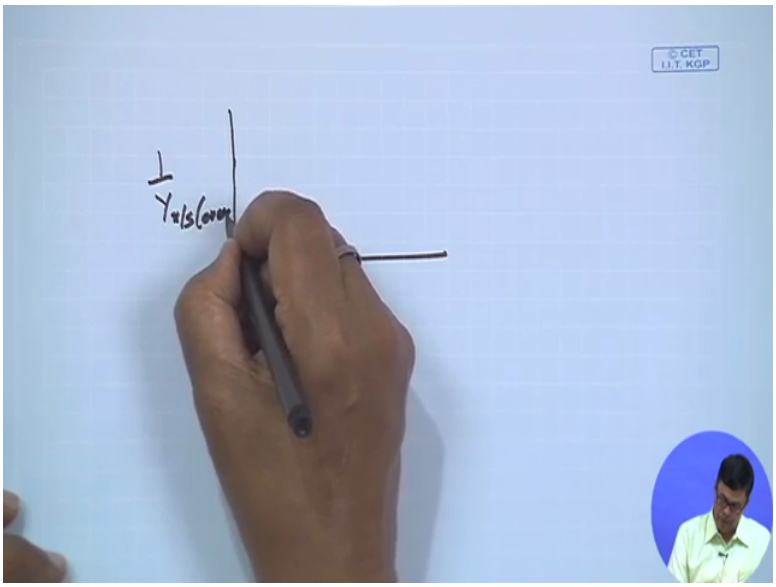

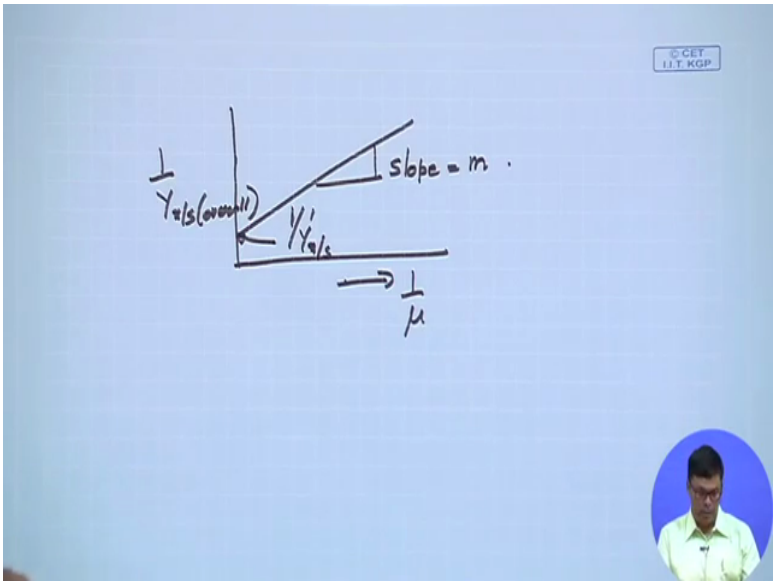

Here we can plot one by meu, you will get a straight line plot the slope will give you the value of m. What you call maintenance coefficient and intercept will give you one by y x by s dash. This is the true growth yield coefficient. True growth yield coefficient this is like this. We can we can easily find it out. There that you know for the cells.

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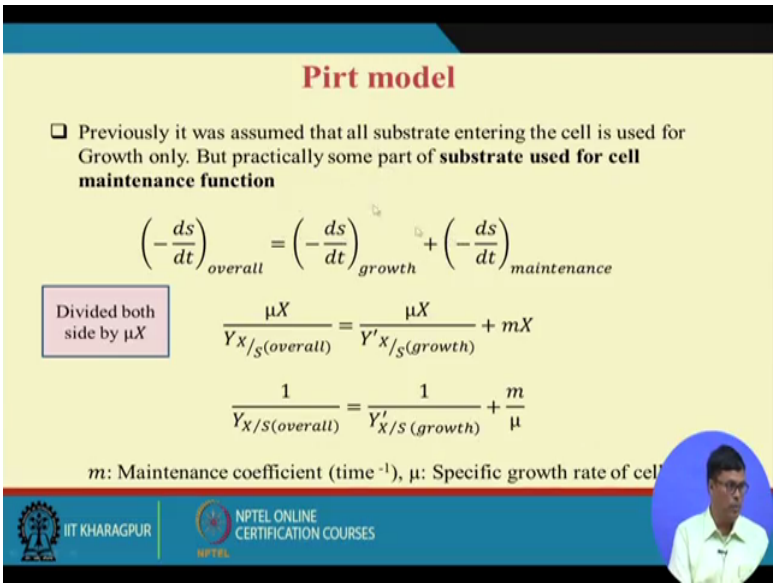

So this is how we can find out the pirt models what model that can be used for finding out the maintenance of the cells. Now let me discuss some problems. First problem that we have chosen that is pseudomonas. You might be heard that about the pseudomonas is very this is the the bacteria which was first genetically modified and is largely in the wastewater treatment process.

The sitters that has the minimum doubling time is 2.4 hours when grown undefeated in a chemistry Corporation that follows the most wanted the k's value is 1.3 gram per litre. Yield coefficient point 4 gram of cell per gram of acetate is there is the initial substrate concentration is 38 gram per litre that means the initial concentration is 38 grams per litre. So what you have to find out the value of a s and x.

S is the steady state substrate concentration. X is the status state mass concentration and dilution rate d equal to half to Dmax. Dmax is I told you in the last class. Dmax is the dilusion rate that when rate of cell mass formation is maximum and also you have to find out the cell mass productivity.

What is called the cell mass productivity? This is how much cell you are producing per unit time that at point 8 into Dmax and you have to calculate also Dwashout out. So this is a very interesting problem. Let us see how you can solve it. Now you can remember that that we we calculated the doubling time.

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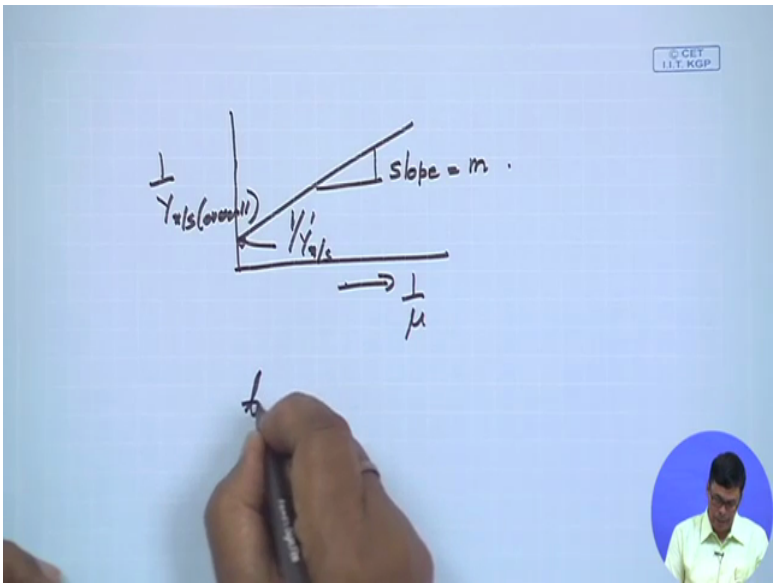

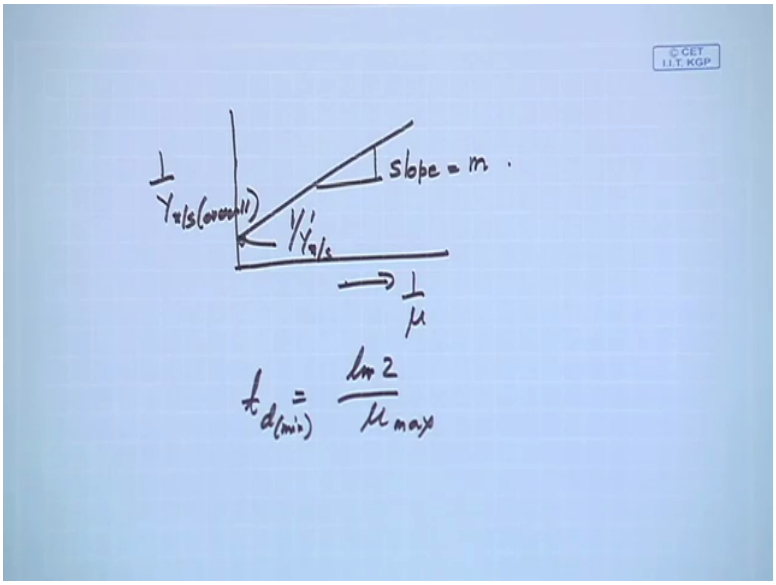

Doubling time how you calculate td equal to lm2 lm2 by meu. Am I right? Meu the minimum doubling time then this will be meu max. So in this problem it is mentioned that suitable has has the minimum doubling time 2.4 hours.

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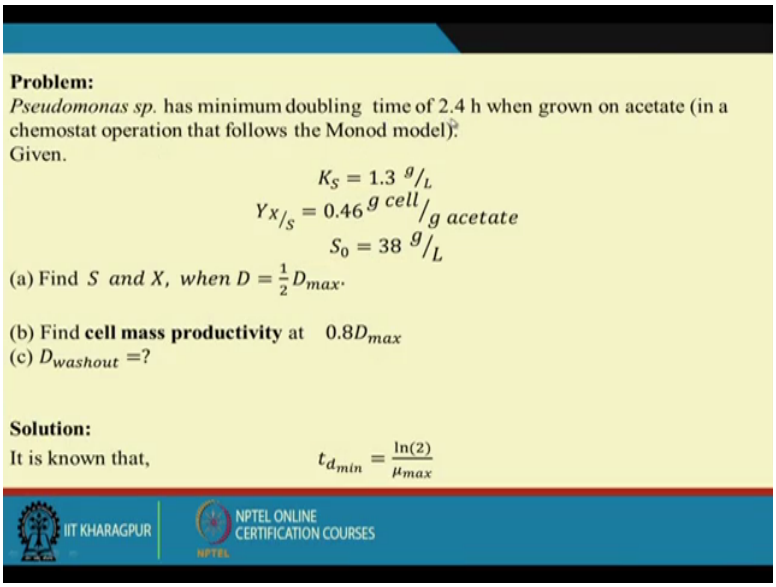

So if it is 2.4 hours then we can easily calculate meu max equal 2.28 hours 288 hour inverse.

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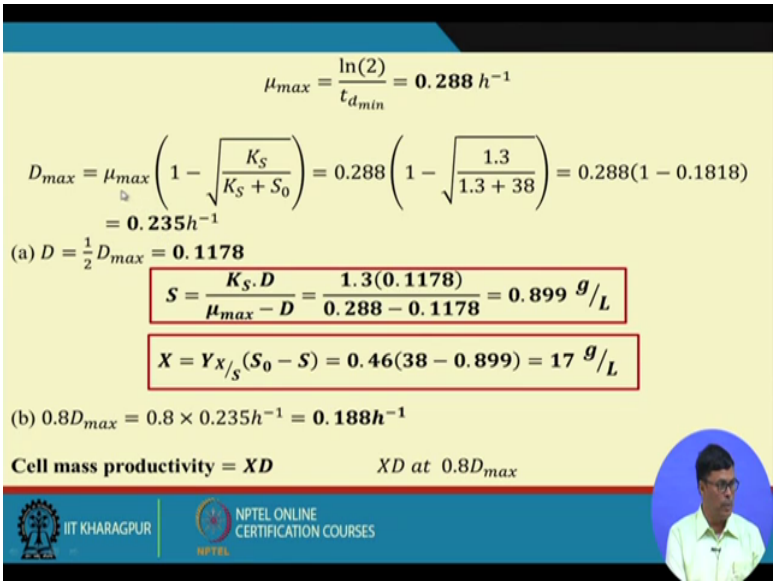

Then Dmax you can calculate with the help of this equation this we derived in the last class. Dmax 1 minus root of a ks by ks + s0. This is equal 2.23 hours inverse. Now in this first part we have written D equal to Dmax. So we can have dilution rate equal to 2.1178. Now we have also find out as steady state code under steady state condition the substrate concentration equal to ks into D, dmax minus D.

So if you put this different value ksd you get the substrate concentration that is point 899 gram per litre. The x you can calculate y x by s S0 minus S and this is equal to 17 gram per litre. So it is very easy to determine the steady state substrate concentration and steady state cell mass concentration in the reactor. Now in the second part dilution rate is point 8 into D Max. D Max value already we calculated. So dilution rate is what point 8 point 188. Now what is the cell mass productivity? Let me show you.

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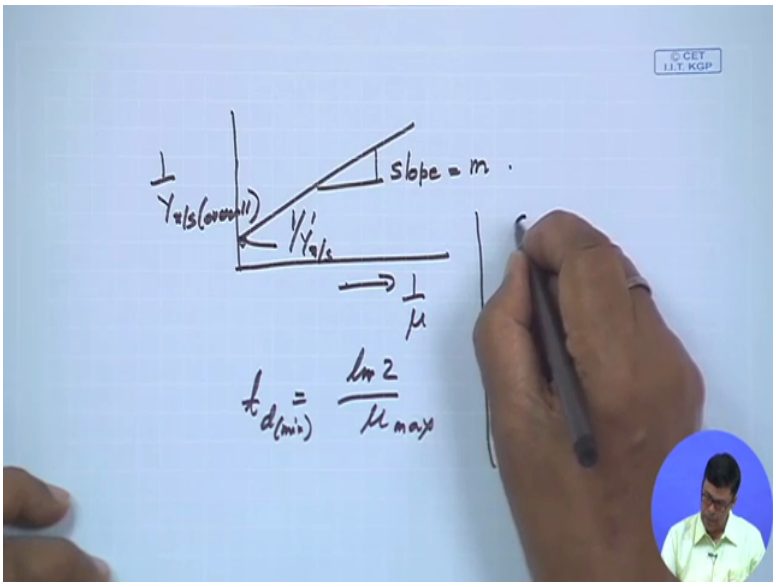

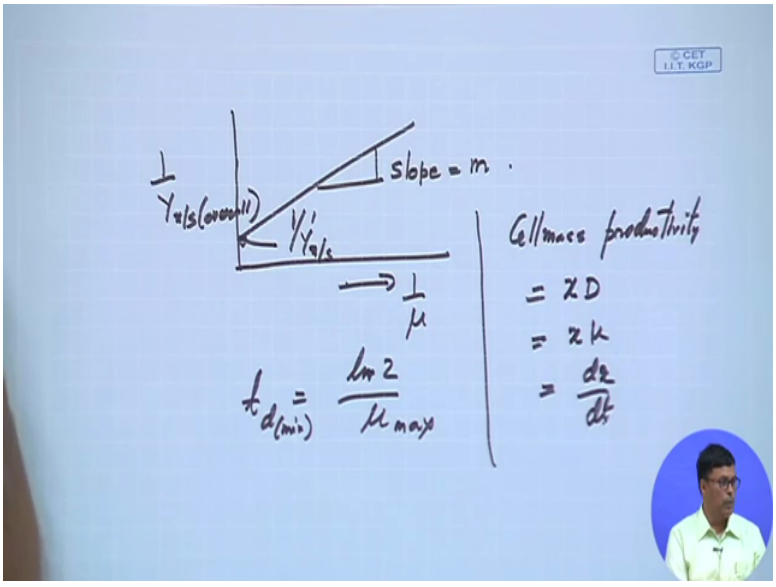

Cell mass productivity is what? Cell mass productivity. Am I right? Productivity is what? Usually it is the body mass cell mass concentration is there multiplied by D. Because we have shown you this is d under steady state condition and d equal to meu. And this is will be equal

to dx by dt. Dx by dt is the mass of cell produced per unit time. So this is exactly that cell mass productivity is equal to X into d.

We have this rate. So you have first we should have to find out what is the cell mass concentration at the solution rate then we can multiply that X with d. We will get the value of cell mass productivity this is exactly they have found out that x we we find out that is 16.35.

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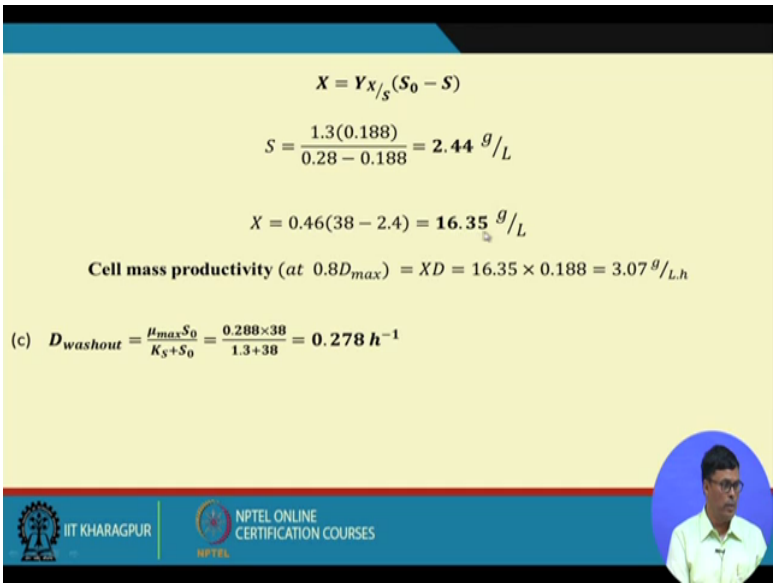

And then u into 14.1 88 we get the cell mass productivity. Its unit is gram per litre per hour. Dwashout again in simple washout x is equal to zero. Since x is equal to zero s will be will be equal to zero. So Dwashout equal to vmax is zero. Ks plus s0 and this is equal 2.279 hours. So this is there so we can easily solve this problem.

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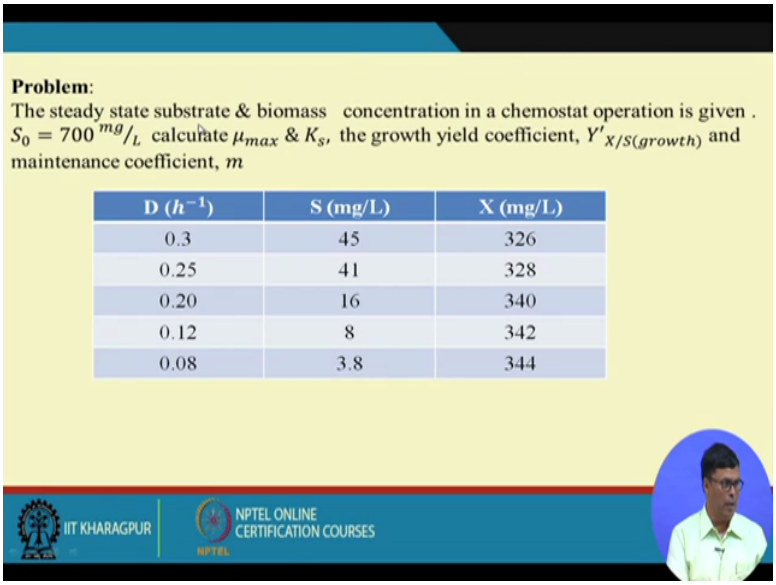

We try to we have another problem with the steady state substrate and Biomass concentration in chemistry operation is given by is s0 equal to 700 millimetre per litre. Calculate calculate Meu Max and KS growth in Coefficient. It will growth yield Coefficient and the maintenance of the maintenance coefficients. So we know that this is not a batch process is a continuous process.

So it is the chemistry Corporation so they have given the different dilution that what is the steady state substrate concentration is given their different paste statuses concentration is there.

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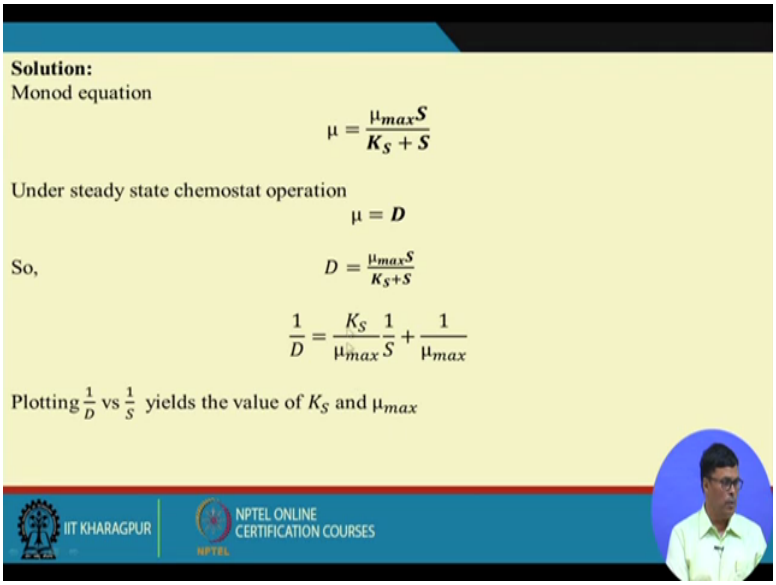

Now this equation reviews the equation we can write in the form of (())(29:00) park Plot. Meu equal to d then I can write one by d equal to ks by meu Max 1 by s plus 1 by Meu Max. If you plot one by d versus one by s plot this is constant and this is constant on the slope you can find out ks by meu Max and intercept we can find out one buy meu max.

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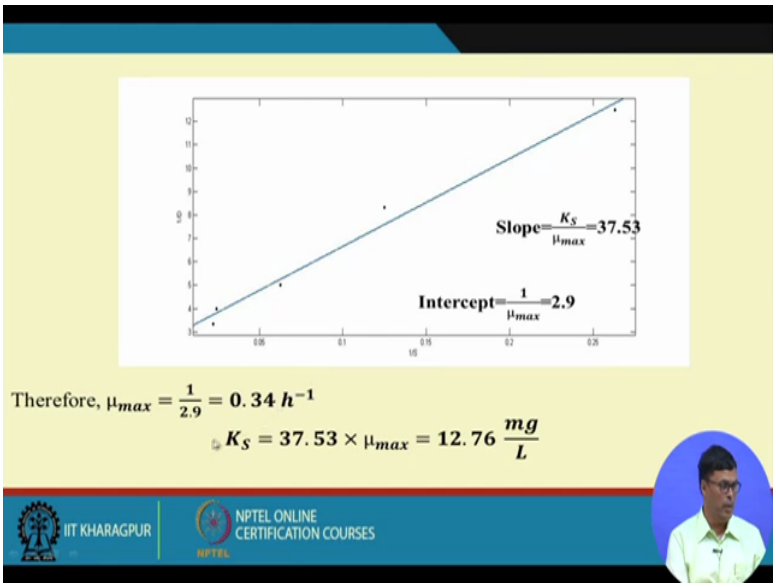

So this is exactly we have found out yet.

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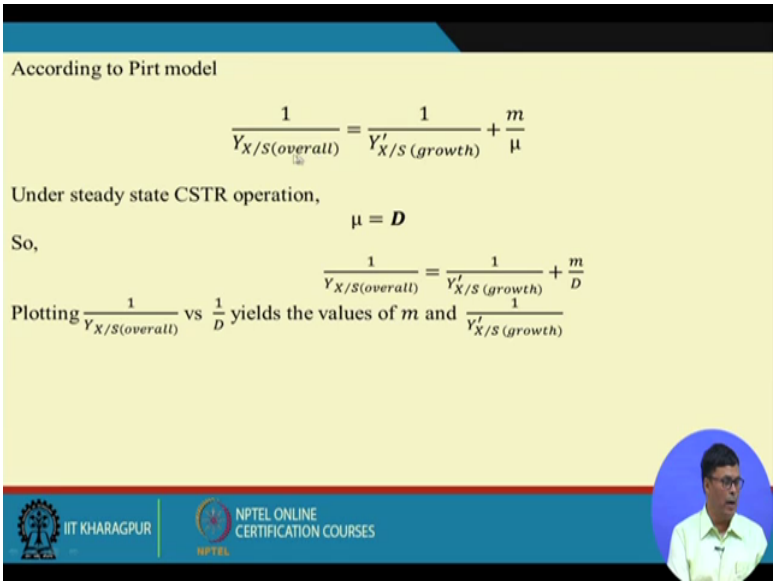

That we Vmax we calculate it and Ks we calculated then according to the pirt model that one by y by by 1 by y dash x by s growth. And under steady state condition meu is equal to v. So we can write this equation like this.

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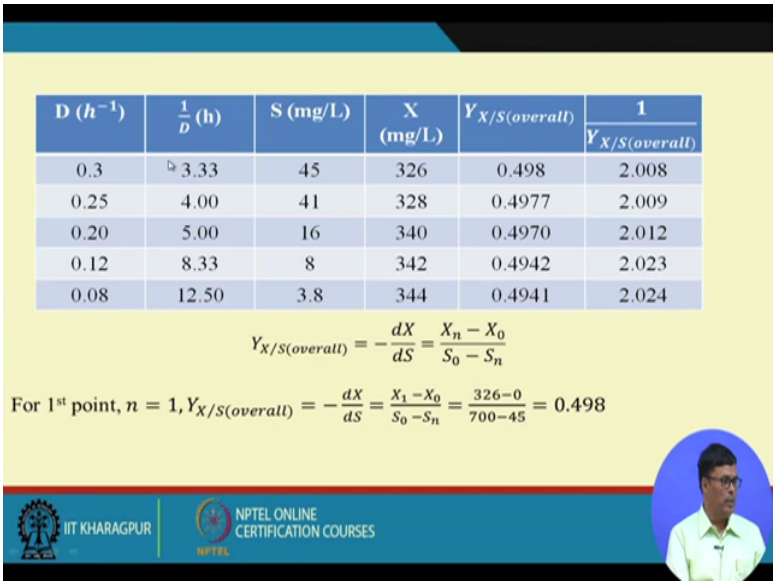

Now in this equation that you see that d is the dilution rate and then this is the this the substrate concentration this the cell mass concentration and from that we can easily find out the coefficient. How we can calculate the inquisition. Inquisition in nothing but dx by ds. We know the initial cell mass concentration and this final Cell mass concentration and initial substrate concentration and final substrate concentration. From that we can find out the inquisition then 1 by a y x by s overall we can calculate.

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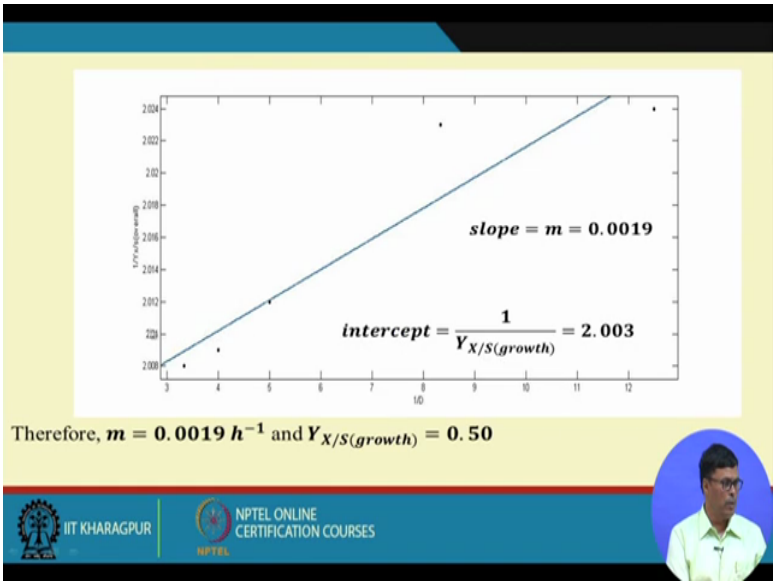

Then we can plot this and from the slope we can find out the maintenance Coefficient. This unit is odd numbers and in the in the intercept we can find out the true growth rate that is about point 5.

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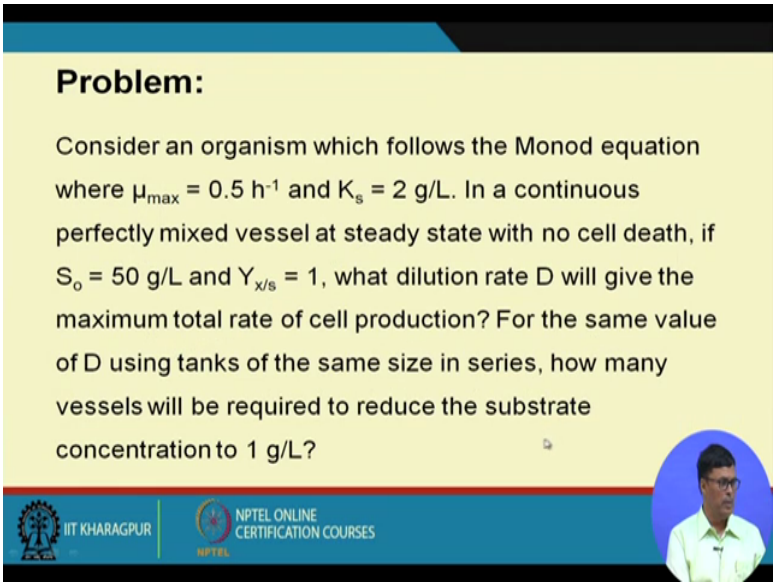

So this is very simple than other problems that I had that I have given as a as a assignment if you this is for this I will solve your over. I will discuss the the year because is fermentation process. Thank you very much!