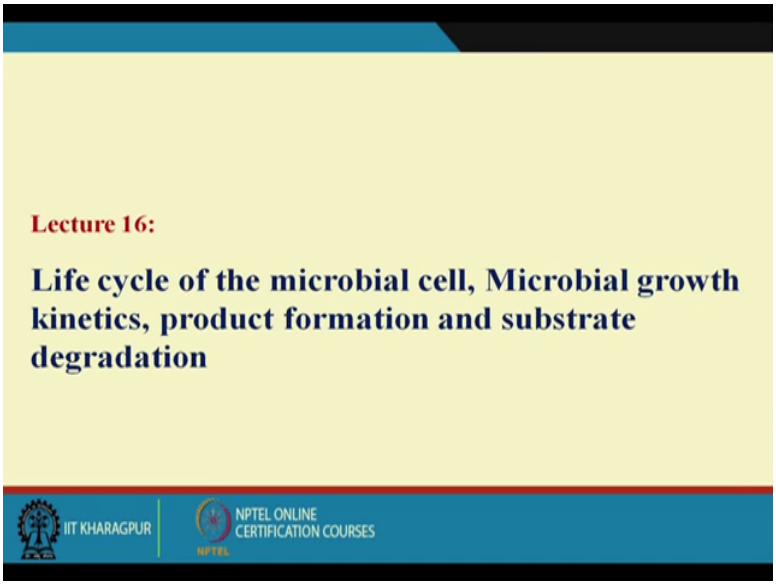
Course on Industrial Biotechnology
By Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology Kharagpur
Lecture 16
Life Cycle Of The Microbial Cell, Microbial Growth Kinetic, Product Formation And Substrate Degration

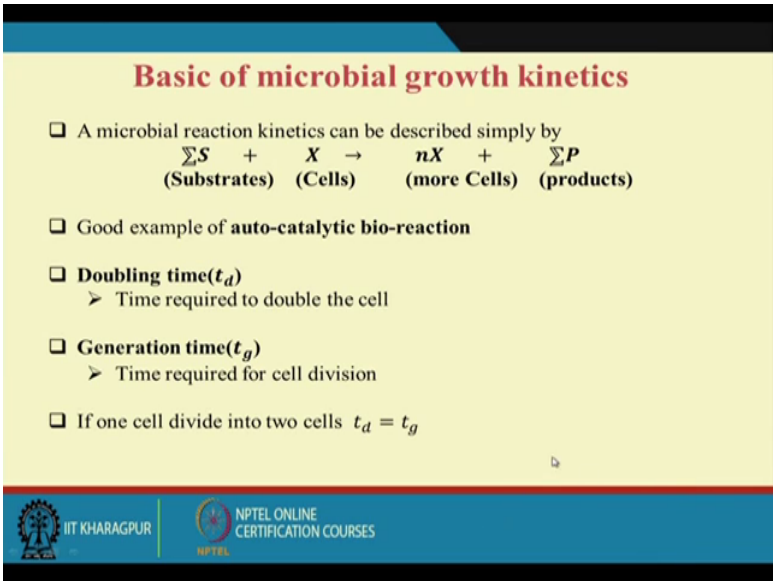
You welcome back to my presentation on industrial biotechnology now today I am going to discuss very important topic this is life cycle of microbial cell.

(Refer Slide Time: 0:32)



And microbial growth kinetics product formation and substrate degradation.

(Refer Slide Time: 0:38)



Now if you look at the basic this microbial growth kinetics so we have substrate then we have cell it produce more cells and if you have other than cells you have product then you have product now in case you have you have the I told you before also in case you don't have product other than cell then it this we can considered as the autocatalytic reaction because it's a very good example of autocatalytic reaction.

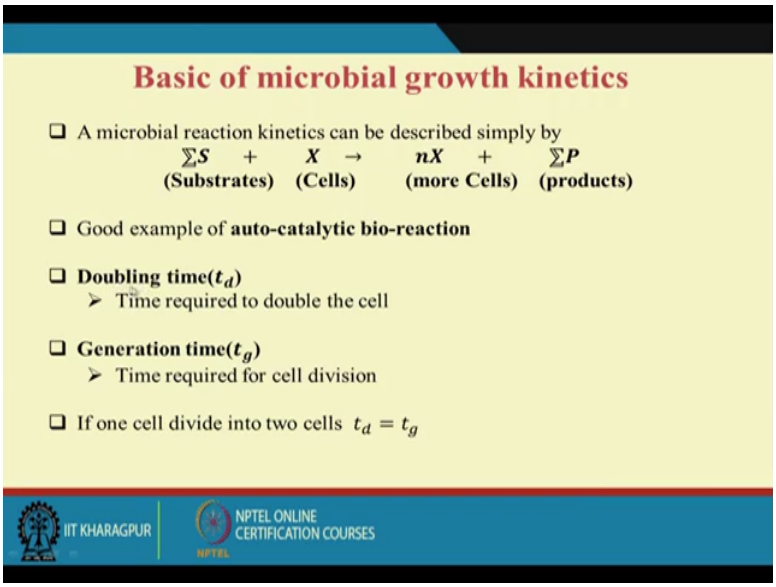
Now here when you cell I told you the cell has different characteristics because living cells as the different characteristics first important characteristics is that a reproduction and second characteristics is that very sensitive to the environment third characteristics is the there acclimatized to the they can be acclimatized to the they can be acclimatized with the different conditions as per example.

That suppose we initiate the organism may not be going at a certain phenol concentration like you know I personally work with a (())(1:52) and when you use the (())(1:55) for phenol degradation we find initially we find the small concentration of phenol will not allowed this organism to grow because it has some germisidle property but when you acclimatized this organism with a little bit higher concentration of phenol.

Then we find that is god for the degradation of icons even high concentration of phenol so acclimatization is very important property of the micro organism then any living system they also discharge some kind of waste product every living system then discharge some kind of waste product that is also very important thing that we have and another thing is that this living system particularly this microbial system.

The special it is that I told you before also from one particular state we can get a number of products we just change the organism and we can have depending on the characteristics of the organism we can get the different products I have given the example of the glucose we can convert it to ethanol with the help of sacramises cyravesia same glucose can be converted into citric acid with the help of aspergesnigar now now then we handle this micro organism.

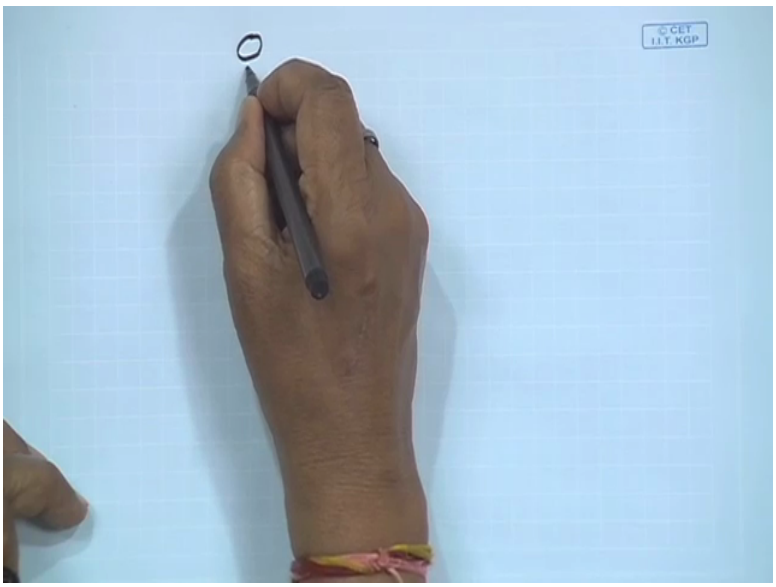
(Refer Slide Time: 3:16)

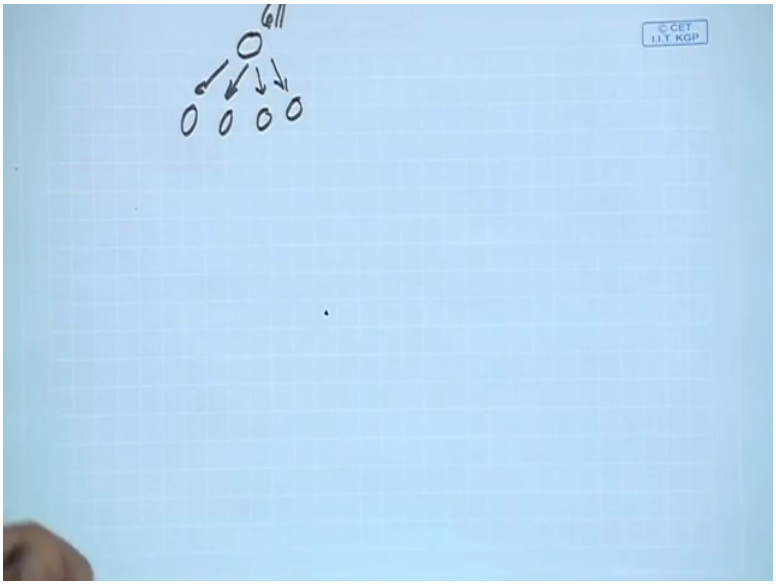


We come across 2 different terms one is called doubling time another is called generation time. Doubling time means time required double the cell population. Double with respect to number, double with respect to mass whatever is there that is called doubling time the generation time is basically that it is the time required for the cell division not necessarily.

The generation time and doubling time they should be close to they will be equal it might be different because it depends on the cell division characteristics I can I can give the example when cell divide.

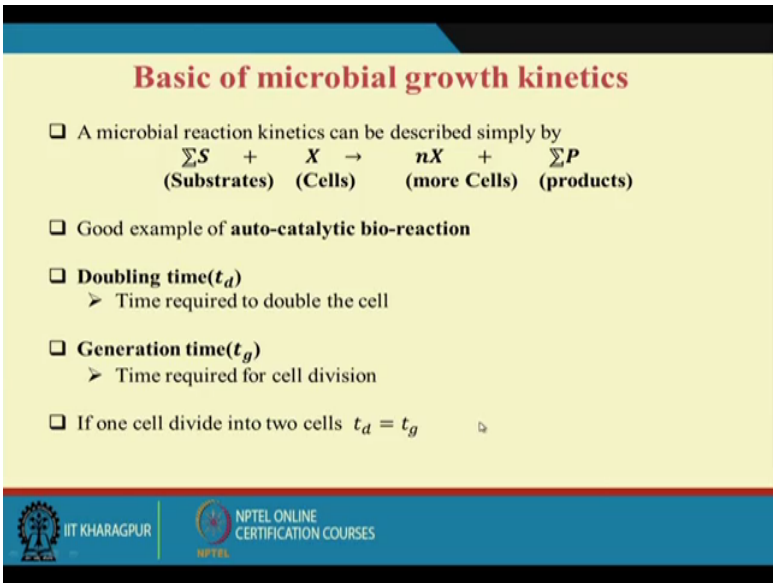
(Refer Slide Time: 3:55)





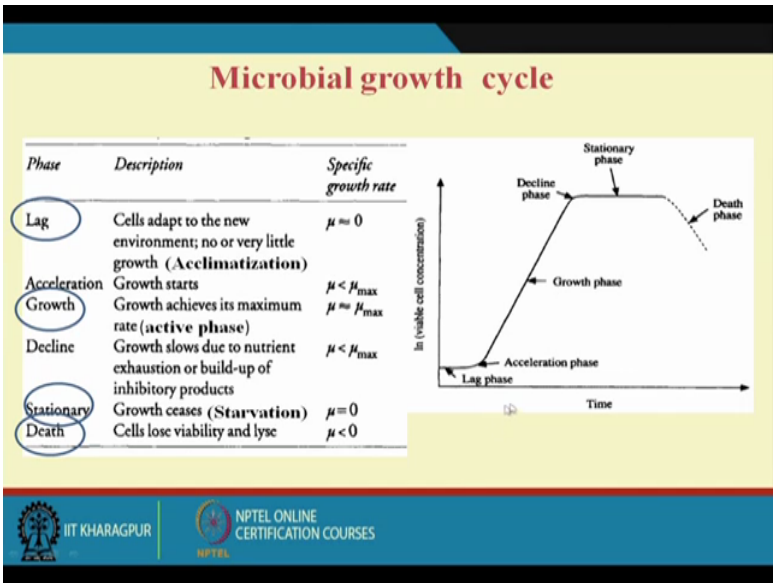
It may divide it may divide into two it may divide into 3 also it may divide into 4 also because it depending on the the characteristics of the of the cells this is the the cell how how they can divide the cell division now if one cell divide into 2 then doubling time is equal to the generation time this is what we have written here.

(Refer Slide Time: 4:19)



When divided into two this doubling time is equal to generation time.

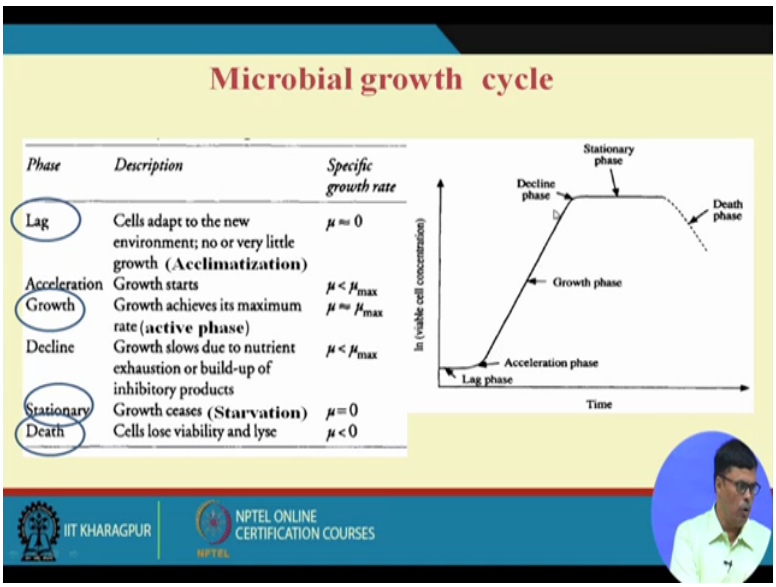
(Refer Slide Time: 4:23)



Now before we go for handling this microorganism it is very essential to know the growth cycle of the organism because why why it is very important because until and unless because organism basically a black box orders we don't know how it is growing the what is the status of the microorganism.

So we should be you should know there status then and only then we can handle the microorganism in a proper way so I can I have because the I can explain this life cycle of the cell how it influence the microbial production process as for example here you see that.

(Refer Slide Time: 5:10)



This the life cycle this is the lag phase this is the log phase this is the stationary phase and this is the death phase now every phase has its significance lag phase is considered acclimatization phase because every organism required some time to acclimatize in a new environment so this this is usually consider as a non productive phase so any kind of organism this phase should be as minimum as possible.

I can give you a typical example in case of sacramises cyravesia that even the growth media doesn't content biotin then lag phase will be higher which is undesirable but if if the media content biotin then this will be reduced that is desirable character this is the same then the time required for the growth of the organism will be reduced to a great extend now second is considered as log phase there.

What you call is growth phase this phase is very very important because why it is important because organism they remain active at this phase whenever we do any kind of (())(6:19) of the cell this phase we have to take into account because because usually the inoculation of the organism is done either from the mid log phase to latte log phase this is a mid log phase and this is the late log phase so in between.

So we insured that whatever organism that inoculate it should be hundred percent active otherwise you will change get the kind of change of characteristics of organism then we have some decline phase here and then it switch over to the stationary phase. The stationary phase is also signifies that the star vision phase why the stationary phase has occurred because here rate of depth of the cell is equal to rate of growth of the cell that is why it is constant.

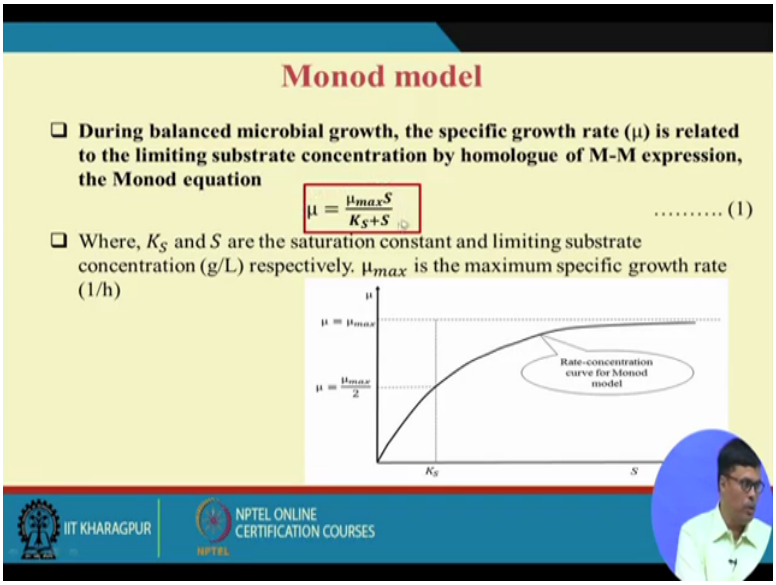
And why it is offering because because due to do the Endogenous respiration of the cells what is endogenous respiration why it is takes place when your substrate amount of substrate present in the media is drastically reduced then the organism the strong organism will try to use the weak organism for there for there who growth in metabolism so here that is why rate of depth is and rate of growth there.

And most of the secondary metabolise formation take place in the fermentation process during this stationary phase the stationary phase the cell physiology is totally different as compare to growth phase so since this is the physiology changes we get the secondary meta and most of the antibiotic I can give the example as per example penicilin (())(7:58) is that is usually produced in the stationary phase.

And what is our objective how to elaborate extend this stationary phase if we extend the stationary phase then we will get more secondary metabolise how we can do that we put the little substrate to the fermentation media so that the stationary phase may be extended and we increase our secondary metabolise formation.

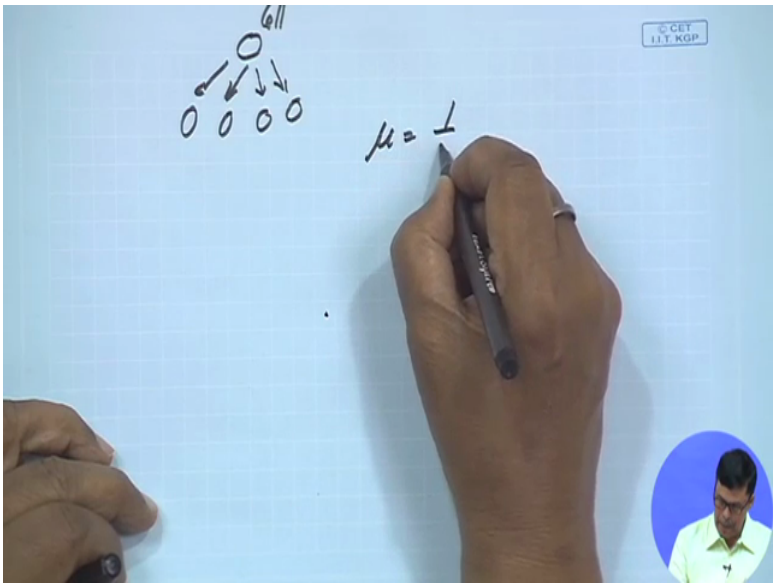
Now these are the phase and this is the dead phase this was the rate of death is more as compare to rate of growth so we are not interested about this phase .now for this the growth characteristics of the organism that is usually express with the help of equation.

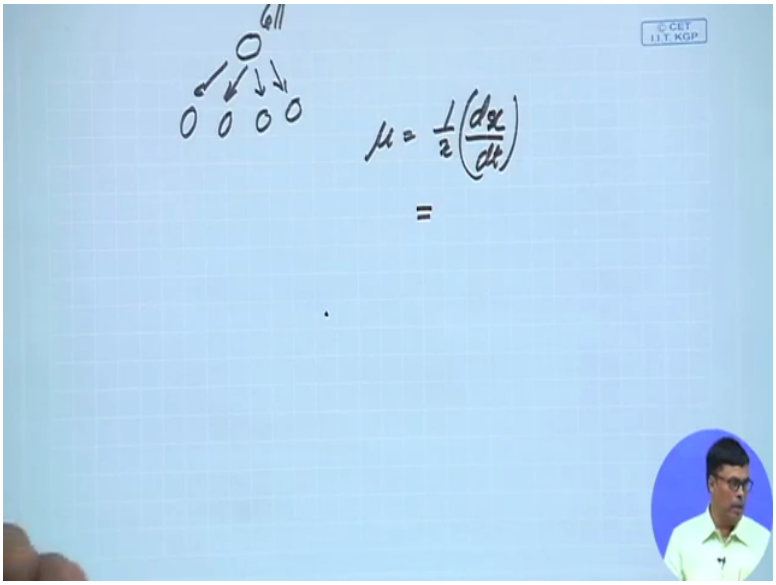
(Refer Slide Time: 8:45)



Similar to Michaels menten expression because during enzymatic reaction we discuss about the Michaels menten equation where V equal to V max is KM plus S now in case of microbial cell growth we use similar type of equation this is Mu equal to Mu max S KS plus S where mu is the specific growth of the cell now what is mu?

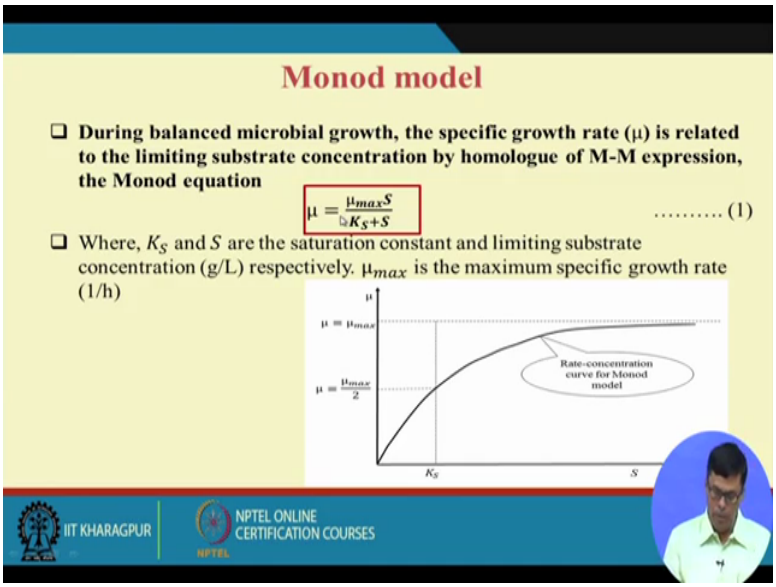
(Refer Slide Time: 9:14)

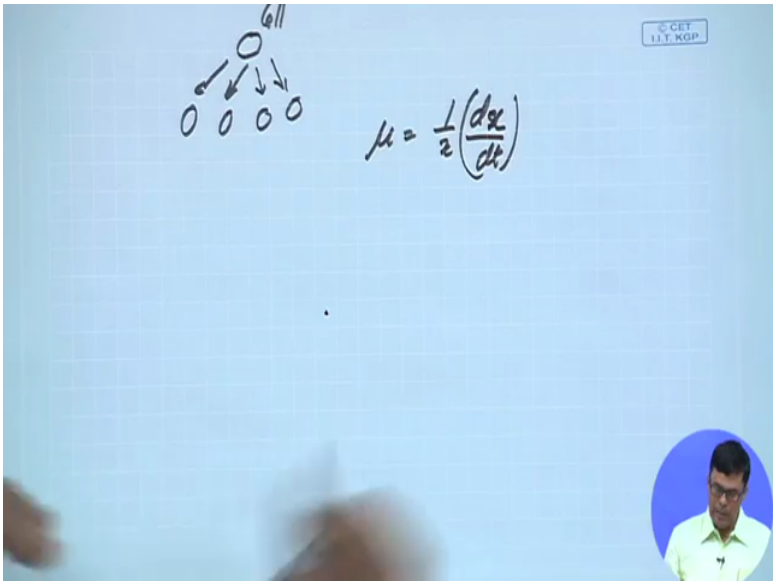


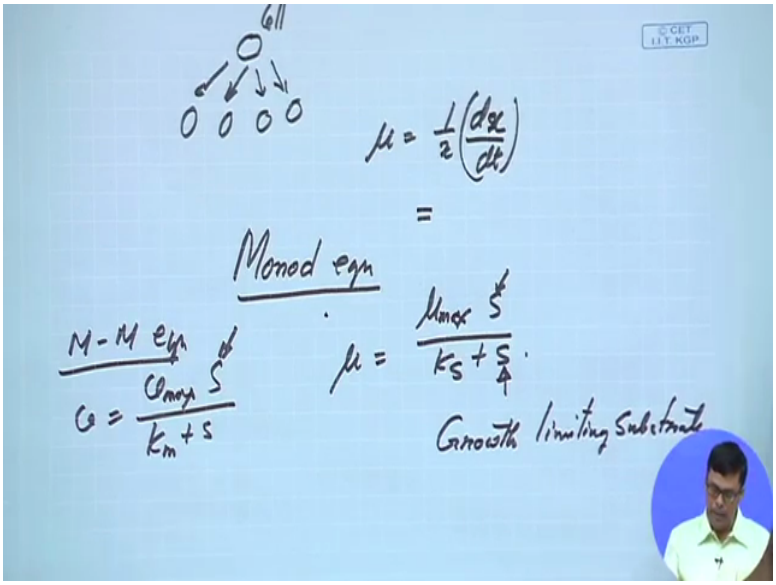


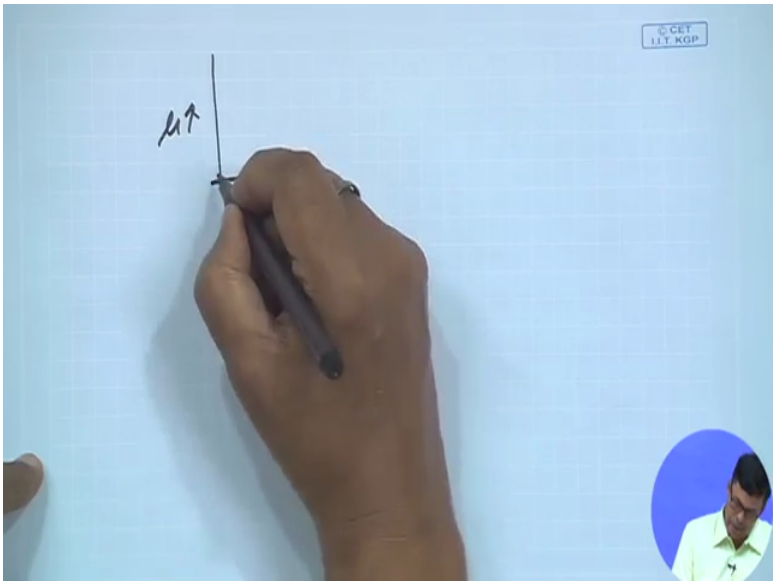
Mu is 1 by X dx by dt what is X? X is the cell mass concentration now it can be define as the rate of cell mass formation per unit cell mass concentration 1 by X is the per unit cell mass concentration so why the specific the specific growth rate means rate of cell mass concentration per unit cell mass concentration this is what there. Now (())(9:46) equation is mu equal to monod equation is what?

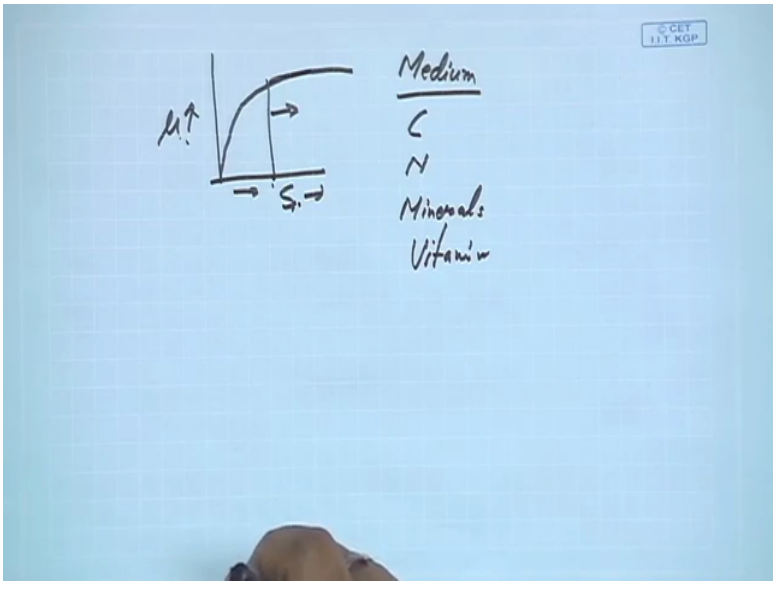
(Refer Slide Time: 9:48)






Cell
$\mu = \frac{1}{x}\left(\frac{dx}{dt}\right)$
=
Monod eqn
M-M eqn
$\mu = \frac{\mu_{max} S}{K_S + S}$
$\mu = \frac{\mu_{max} S}{K_m + S}$
Growth limiting Substrate


$\mu \uparrow$


$\mu \uparrow$
$S \rightarrow$
Medium
C
N
Minerals
Vitamin

Monod equation Mu equal to mu max S KS plus S. Now I can I can simultaneously I can write the Michaels menten equation here so that you can find the similarity this is equal to V max S Km plus S. So now here this S and this S is the consider as the this called the substrate the in case of enzymatic reaction the your substrate is very specific to the enzyme am I right? So as per example that in case of glucose iso isomeric reaction.

The substrate is glucose but in case of microbial growth this is consider as the growth limiting substrate growth limiting substrate. Now what do you mean by growth limiting substrate that means the you see that suppose you have mu and S it is like this so up to this you as you as you change your S value your mu changes and after that it remains flatten. So above this this is independant mu is independent of S constant to concentration of S.

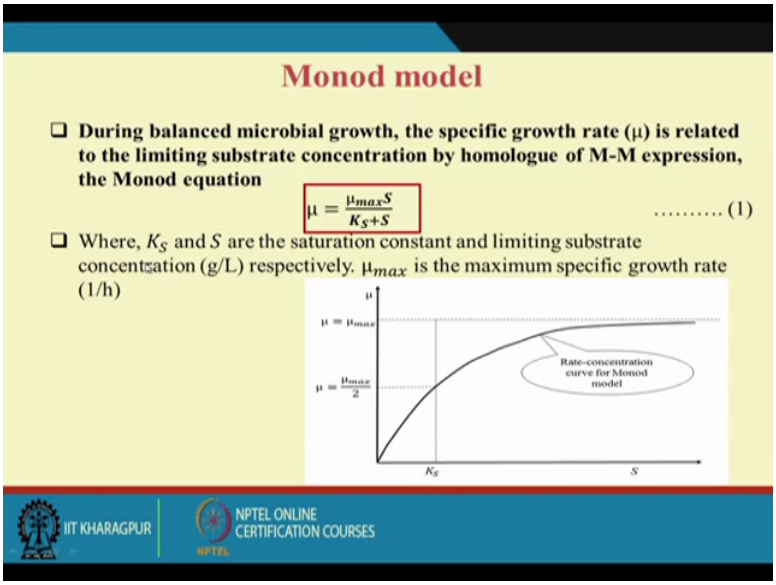
But below this value your mu depends on the concentration of S. Now if you if you I told you in the medium the group media you have different components you have carbon source you have nitrogen source you have minerals and you have vitamins. So different components are present in the in the formal media now suppose you keep other other all materials in access and concentration of C carbon you change from 0 to infinity.

And try to find out that whether the mu changes with respect to carbon source or not and if you find that is change is with respect to carbon source so we call it growth limiting substrate. Now suppose we want to find out the effect of nitrogen we keep carbon, mineral and vitamin excess so that excess means the whatever required in the media we keep it in excess so that there should not be any if should not be limiting factor for the growth of the organism.

And concentration of nitrogen source you change from 0 to infinity and if you find your mu value changes with respect to concentration of nitrogen source then we call it in the growth limiting now if mu value doesn't change with respect to nitrogen source is remain constant then it is not a growth limiting substrate same thing happens to the minerals and vitamins so what I want to emphasis here in case of enzymatic reaction.

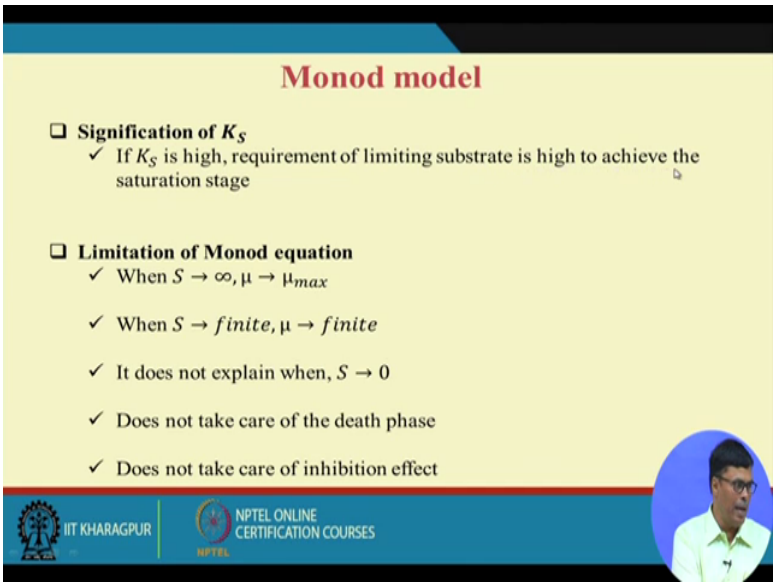
S is the substrate with respect to enzyme which is very specific but in case of microbial system the S is the growth limiting substrate and it can be can e anything it can be carbon source it can be nitrogen source it can be minerals it can be vitamins it can be anything that we have so this we should have to keep in mind.

(Refer Slide Time: 13:33)



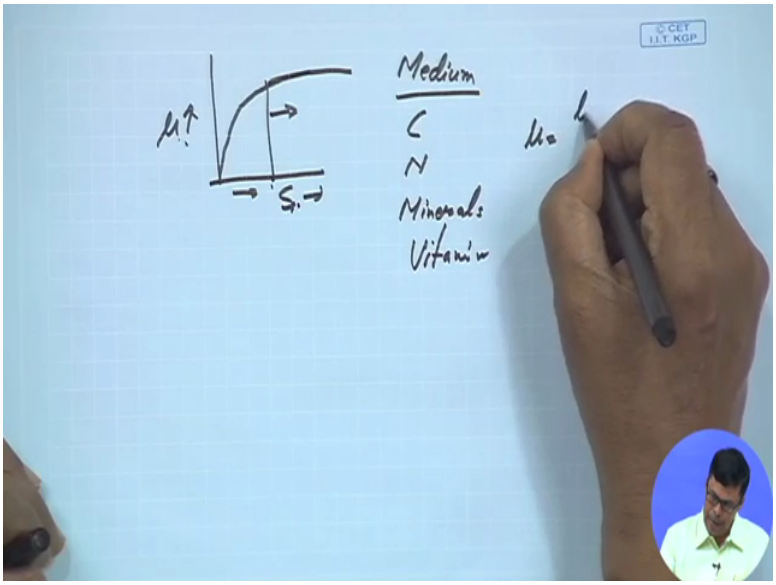
Now we have come across in (())(13:35) in the KS KS is the saturation constant you can see here is the KS that is the saturation constant.

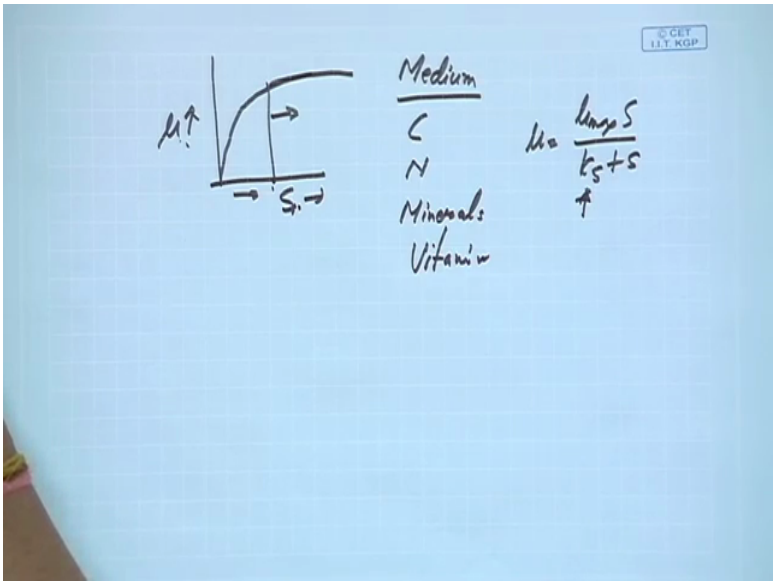
(Refer Slide Time: 13:42)



Now that significance of the case is that if case is high requirement of the limiting substrate is high to achieve the saturated state that is there because you know that is that is very simple .

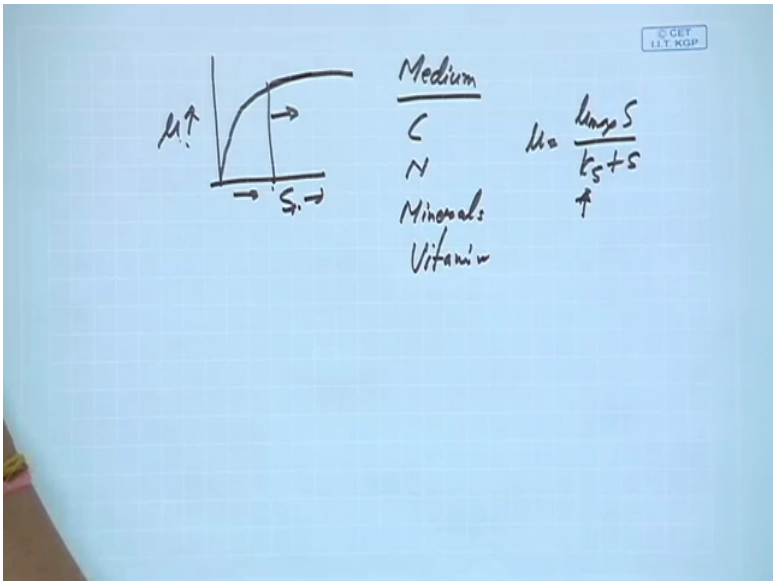
(Refer Slide Time: 14:00)

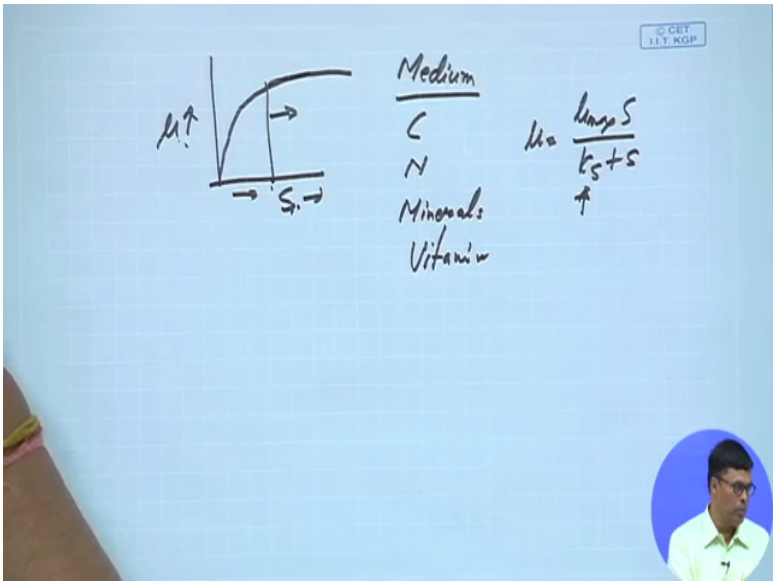




If you mu equal to mu Max S KS plus S now if KS is high then we required more substrate to get a more get the desired amount of cell mass formation so we KS is low that means you required less amount of substrate to get the desired amount of growth of the cells now what are the limitations of the Michaels menten equation.

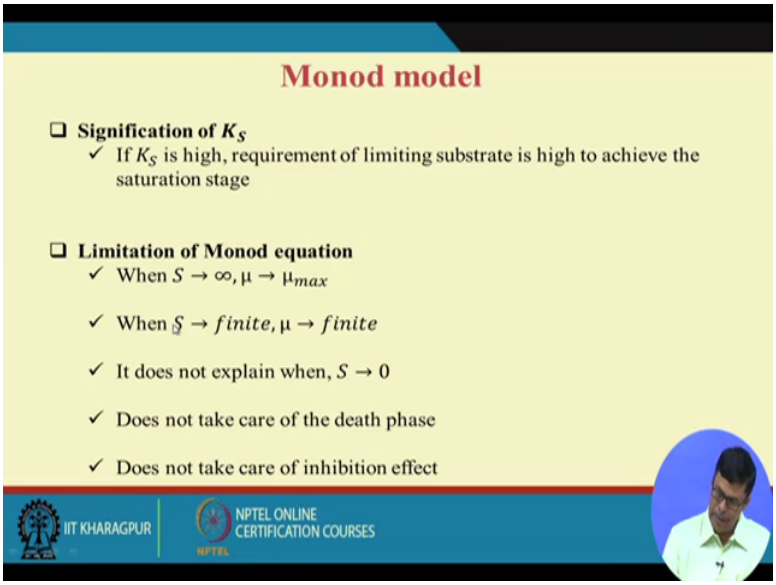
(Refer Slide Time: 14:25)





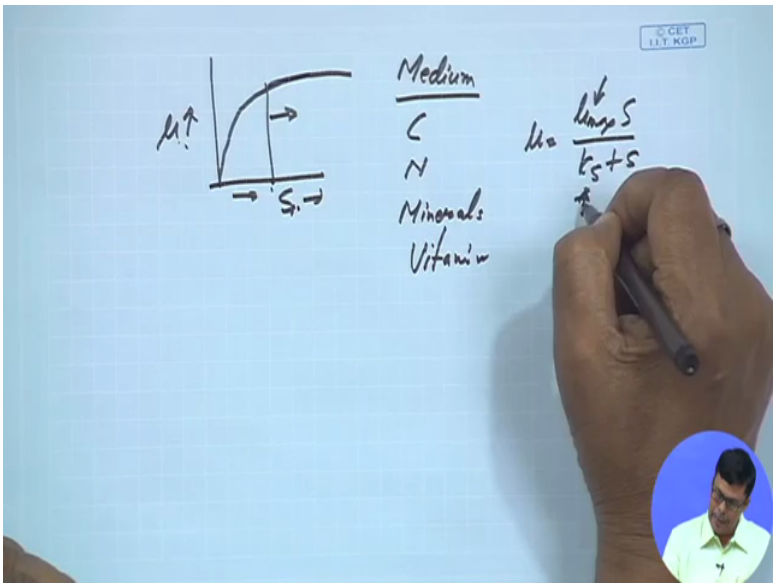
That if S is Infinity suppose S S is the infinity then I can ignore this the with respect to S and mu will be tensed to mu max this is what exactly written when S is intense into Infinity mu tends to mu max.

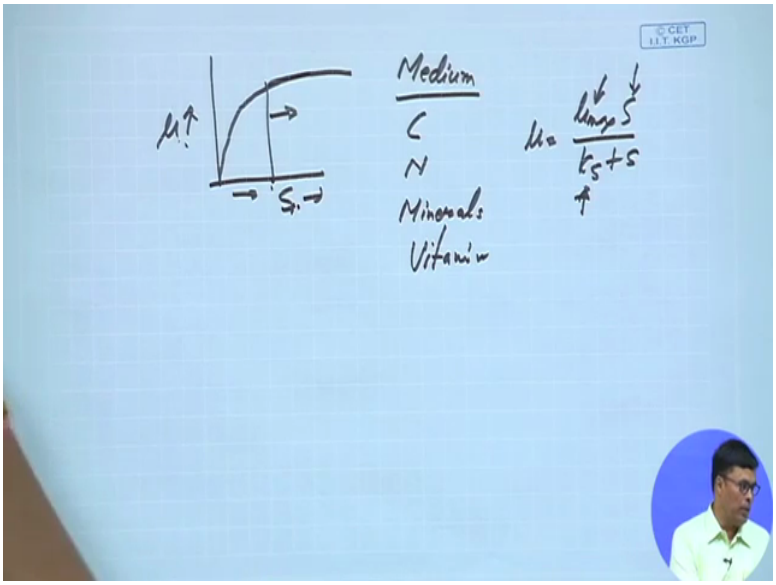
(Refer Slide Time: 14:43)



Now another thing is that if here K.

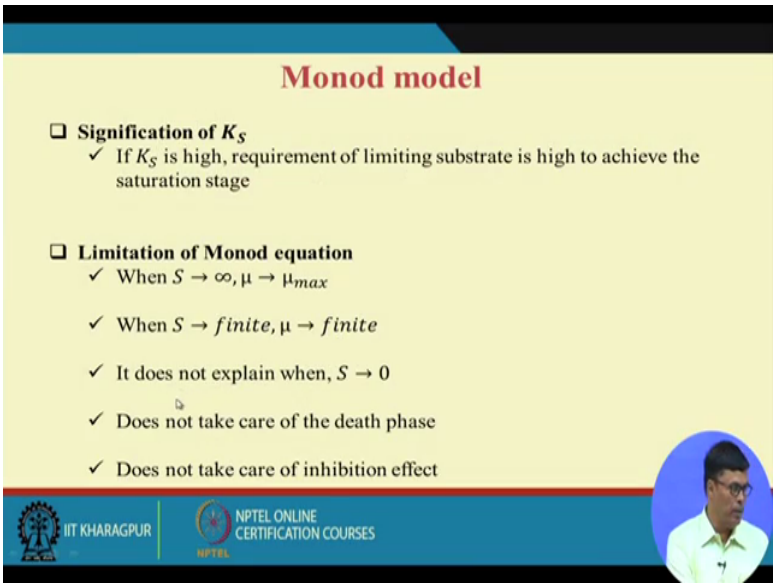
(Refer Slide Time: 14:47)





Mu max and the KS at the constant now if S is finite it has the finite value the mu also should have the finite value so what I have written here.

(Refer Slide Time: 14:58)

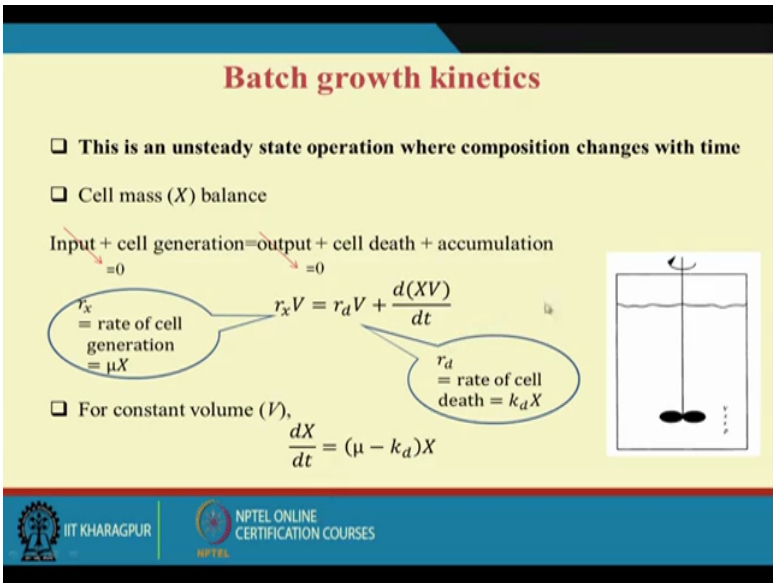


S is finite and mu should be finite now it doesn't explain the situation what will happen when S tends to zero. Because that is that situation doesn't explained here and your this equation also doesn't take care the death of the cell and whenever we handle any kind of living organism than we know in a population always there will be some growth of the cell and there will be the death of the cell.

So that is the normal situation that we have now that the death of the cells we have not cause the Michaels the monode that inconsider the death of the cells another thing they have they also consider that is inhibition effect of substrate and product that is quiet common to the microbial system that you know if you keep on increasing thee the substrate concentration after some time the growth rate will be (())(15:51).

And if your product formation increases then also we will find that after sometime the growth of the organism have purch. This equation doesn't take care this this is the limitations of the monode equation.

(Refer Slide Time: 16:07)

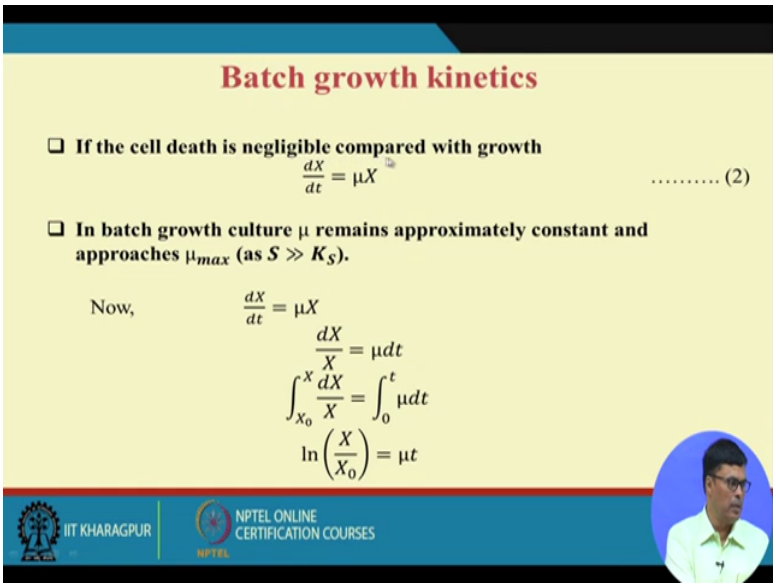


Now if you how you can calculate the rate of growth of the cells now this is the batch process we know batch process we take the media then we take the cell now again I am telling you that when you do the inoculation we should do the inoculation of the cell in meet log phase in between log phase to late log Phase then then then and only then we can get the same result desired results otherwise sometimes we get some kind of (())(16:39) results.

Now here in the batch process there is input and output that should be equal to zero so red cells gen cell formation we can write RX into V Rx is the DX by DT and DX means cell concentration per unit volume so you consider the whole volume so you multiplied by V and this is the rate of death of the cell in a whole volume that AU multiplied by V we will get the total cell how much cell is dying in the whole volume.

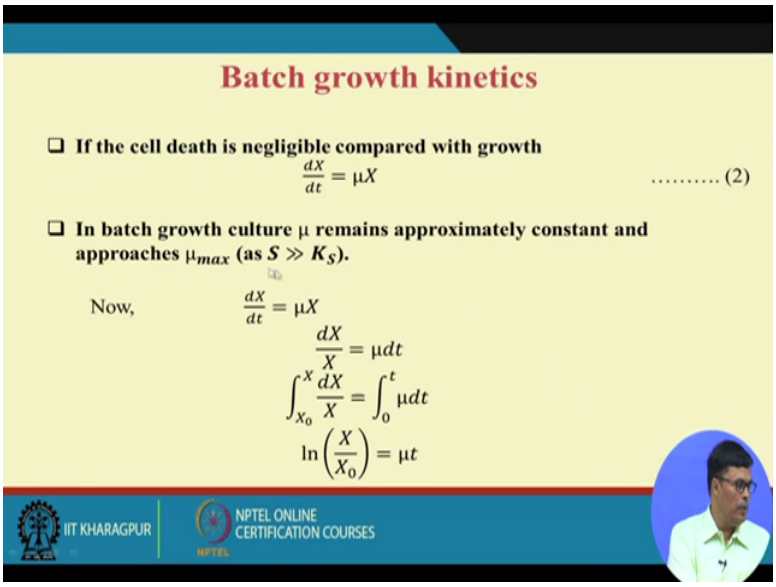
And this is the rate of accumulation of the cell in the system so this this you can if you re write you will gave come across this equation that is the would 1D DX by DT this is the rate of sale marks for accumulation in the system is equal to mu minus KD KD of the specific group death of the cell into X.

(Refer Slide Time: 17:34)



So if the specific that is negligible as compared to the growth of the cell then we can write the one by DX by DT equal to mu into X the here you see this is a KU considered as zero no death off the cell then equation will be DX by DT equal to mu into X then mu will be 1 by X DX by DT.

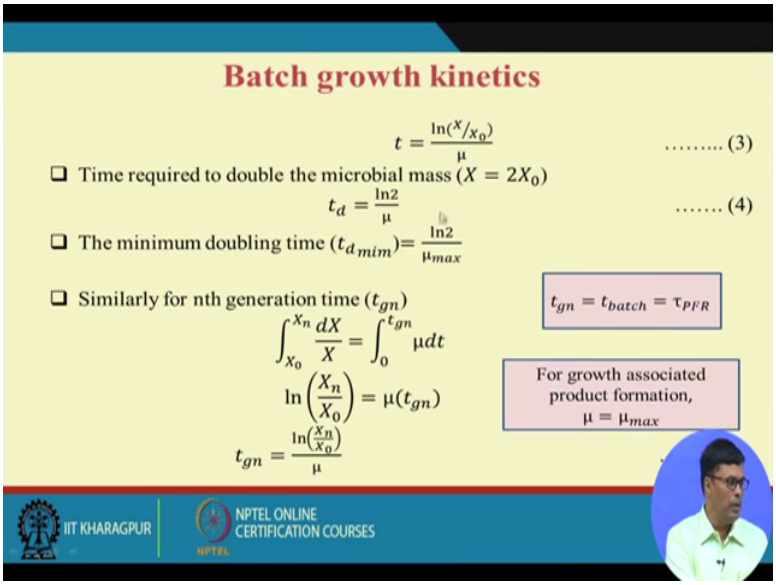
(Refer Slide Time: 17:59)

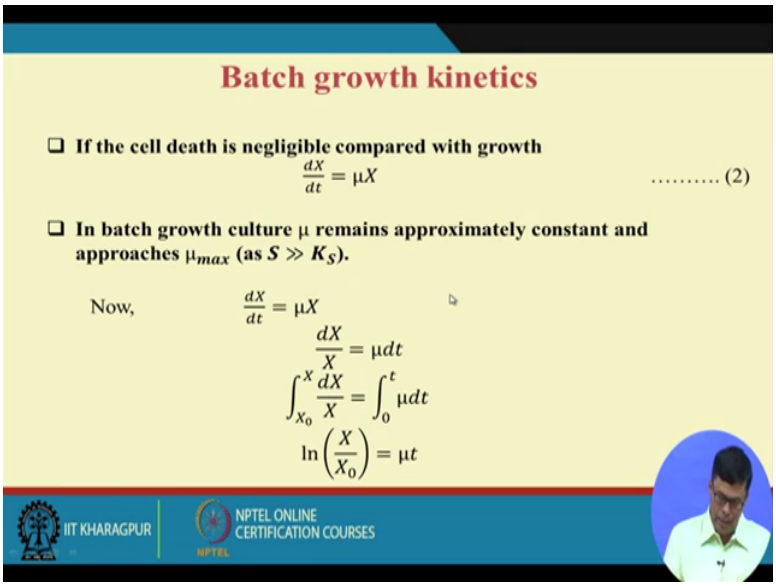


Now when in the in batch growth the mu remains approximately constant and approaches to mu max this is like this and we can we can if we if we assume mu is constant particularly if we assume that your organisms is growing in the log Phase where the rate of growth is more or less constant then we can we can have you come across this the equation that we can we

can integrate that X zero XZ the initial cell mass concentration this is the final cell mass concentration and this is Ln X by X0 equal to mu into X .

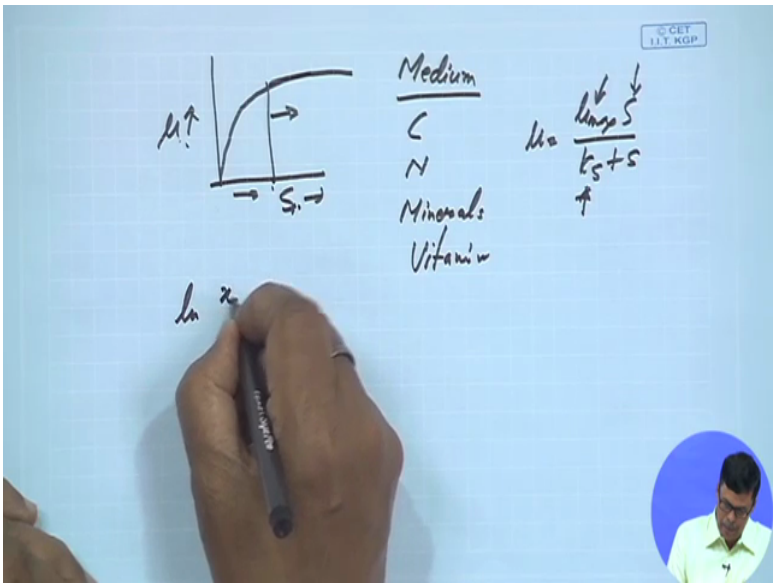
(Refer Slide Time: 18:35)

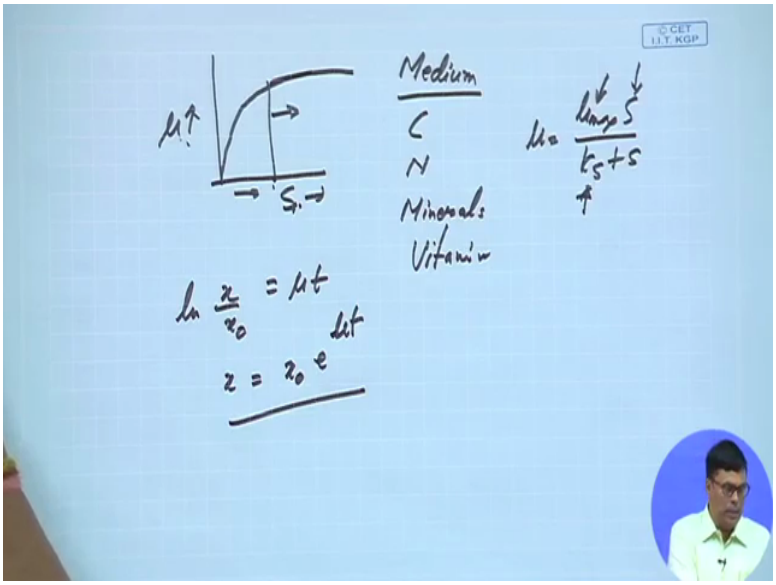




So X equal to then if you if you do this one then we can here we can easily write the if you write Ln.

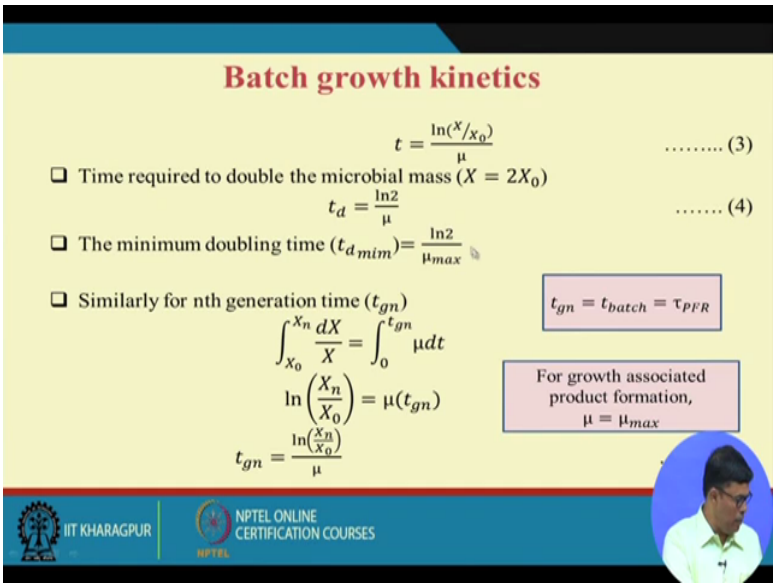
(Refer Slide Time: 18:46)

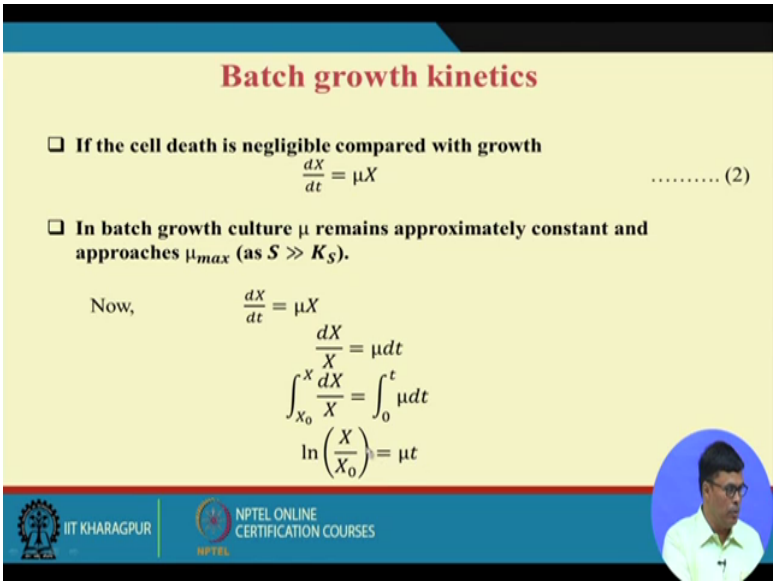




X by X zero equal to mu into X mu into T then X we can write the X 0 into E to the power mu T this we can write this is the cell mass concentration at any time T we can easily calculate.

(Refer Slide Time: 19:05)





So T also we can calculate like this in other way if you see here T also you can you take the mu the denominator you will get that and then doubling time is the as we know that the doubling time is what when the cell will be double that mean X will be 2X0. Initial concentration will be X zero then final concentration to be 2X0 then X 0 X0 will cancel LN 2.

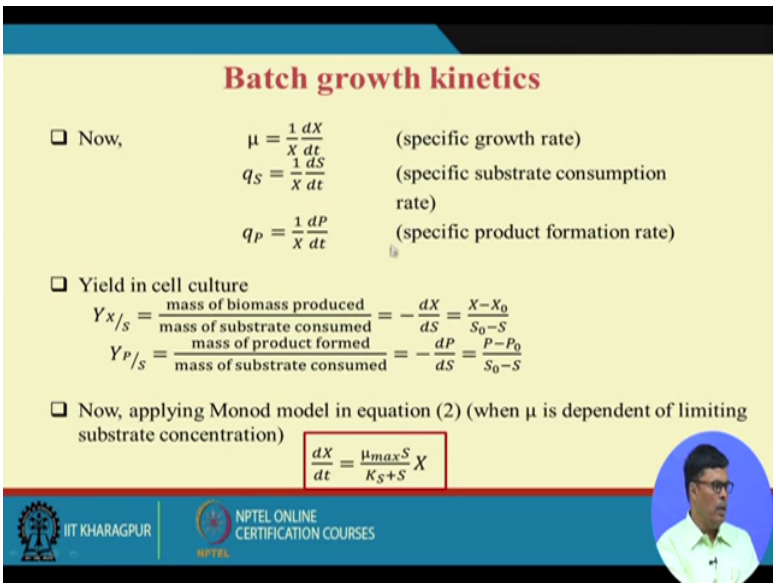
So doubling time is nothing but Ln 2 by mu you can easily calculate now question comes what is the minimum doubling time minimum doubling time will be when you mu Ln 2 by mu Max because this mu Max is the maximum specific growth rate of the cells if you divide

by them we will get so any any problems suppose I give you this it is mentioned that minimum doubling time.

So you can easily find out the value of mu max of the cell that is very simple and then what is the how you calculate the generation time generation time you don't know how much cell is produced lat us the assume X is 0 is convert to Xn then this is Xn by LN Xn by X0 and mu into Fn this is TGN is the Generation time.

Now Tgn genenration time Ln Xn by X0 divide my mu so we can calculate both the Dubbing time and the generation time with the help of this equation.

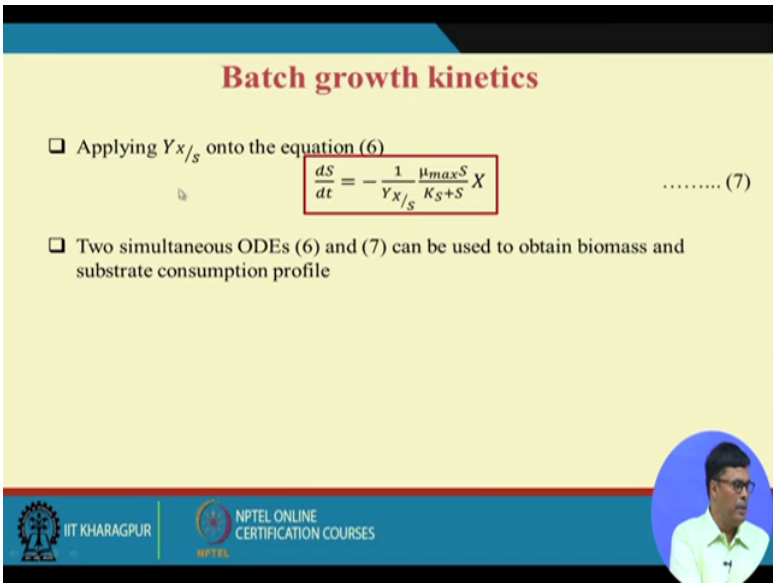
(Refer Slide Time: 20:35)



Now here because I was talking about the specific growth rate can be defined as mu equal to rate of growth of the cell per unit cell mass concentration now similarly specific substrate consumption rate how we can express that DS by DT Ds by DT is the rate of substrate consumption rate per unit cell mass concentration then it is called a specific substrate consumption rate specific product formation rate DP by DT is the product formation rate.

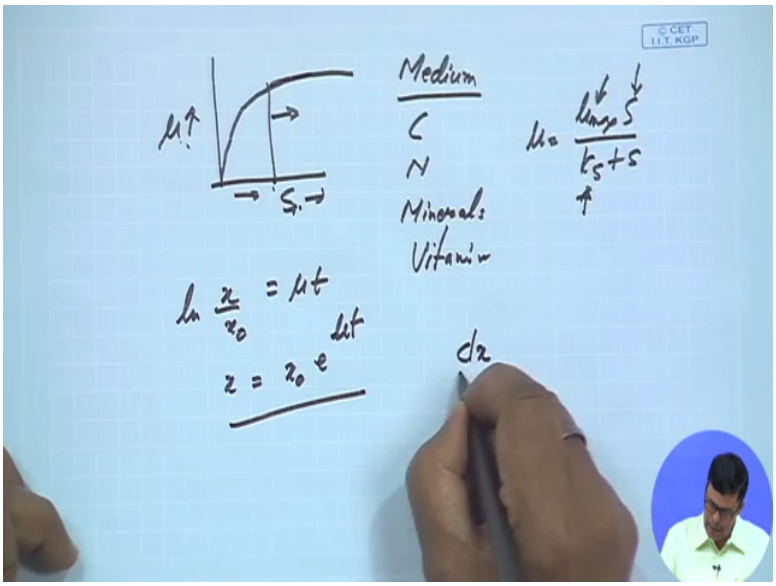
And if you use (())(21:09) specific then per unit cell mass production per unit cell mass how much is the product formation that is called specific product formation right. Now why X by S the yield position you can calculate is X minus X zero is zero minus S and product till you can calculate P minus P0 S minus S zero and this is monod equation we can you put this then DX by Dt we can write this this way.

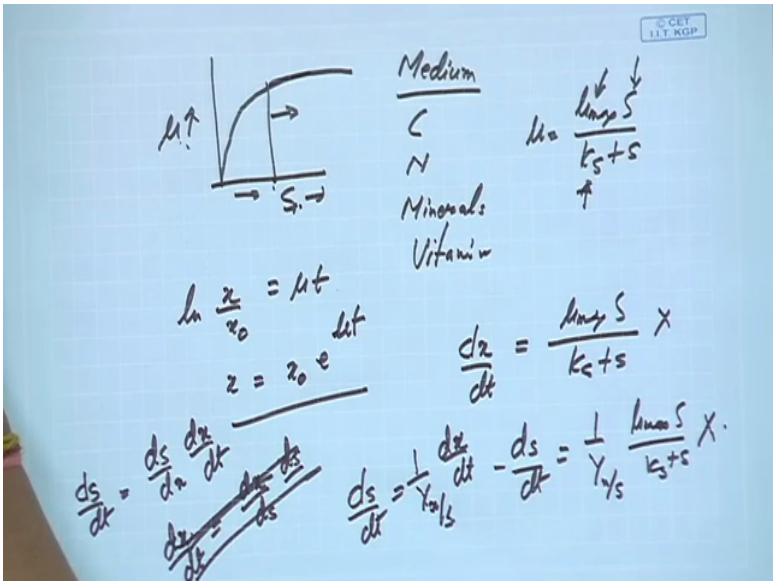
(Refer Slide Time: 21:42)



And rate of substrate consumption you can easily find out how we can find out the rate of substrate suppose if I if I if I take this equation as the account.

(Refer Slide Time: 21:54)

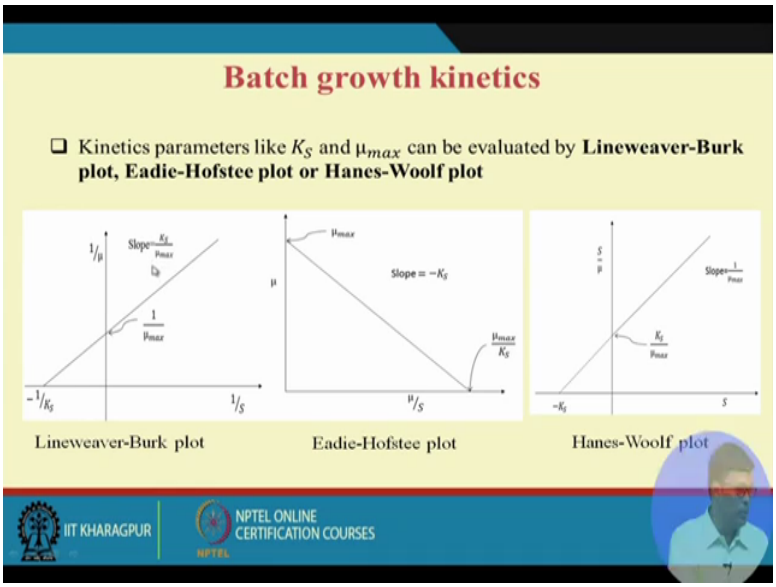




DX by DT equal to mu max S KS plus S into X am I right now how you can find out the DS by DT I can write the DS by DT equal to S by DX plus DX by DT so DX by DT is equal to what I can write the DX by DS by DS by DT no sorry this is not like this then I can write this is equal to DS by DT is equal to one by YX by S because this this is equal to DX by DT so you can come to the denominator.

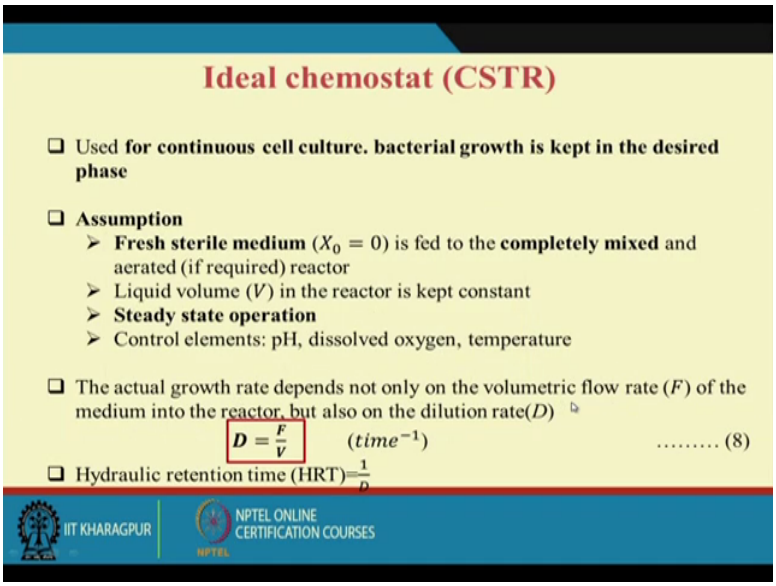
And this is DX by DT so so if you multiply that means DS by DT minus DS by DT you will be a what this is equal to I just you multiply 1 YX by S mu Max S KS plus S into X that is exactly we have done here you can this is the equation we can easily find out so then we have shown you during the enzymatic reaction planet X how to find out the Kinetic constant like km and Vmax.

(Refer Slide Time: 23:32)



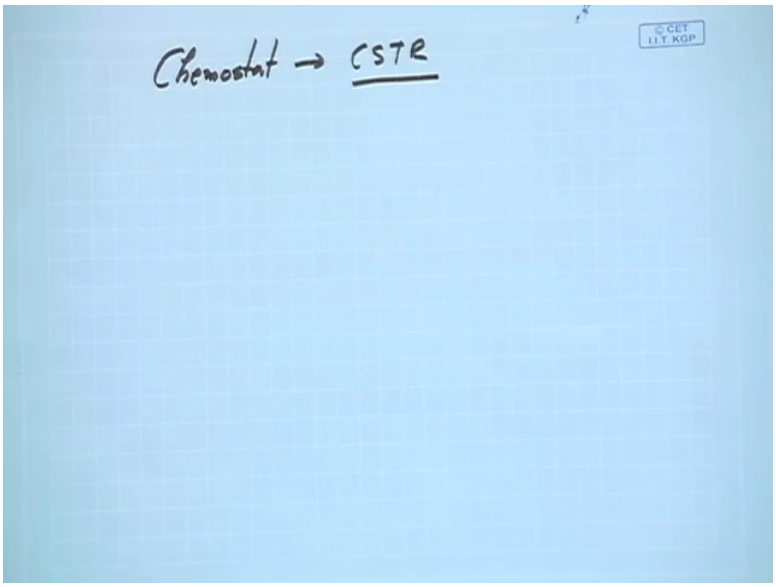
Similarly KM and Vmax here also with the help lineweaver-burk plot, eadie-hofstee plot or hanes-woolf plot we can find out the value of KS and V Max.

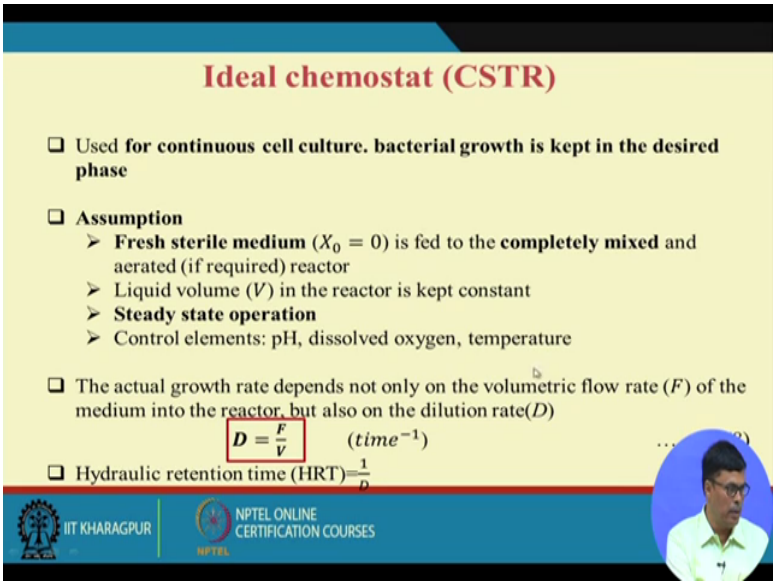
(Refer Slide Time: 23:45)



Now let us go to the chemostat process but let me tell you what do you mean by chemostat chemostat means it is basically this is considered as the CSTR when in the in the chemical Industries we use the term the CSTR continuous stir tank reactor when that same reactor we used in the biological process.

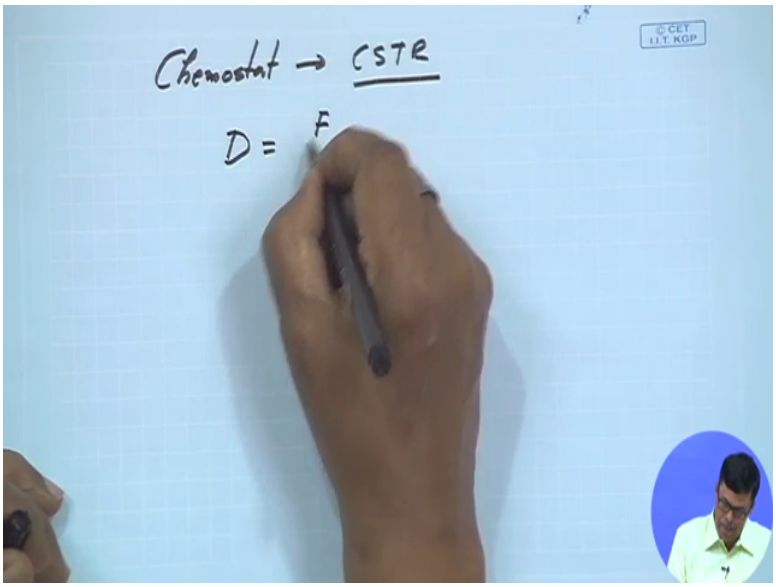
(Refer Slide Time: 24:10)

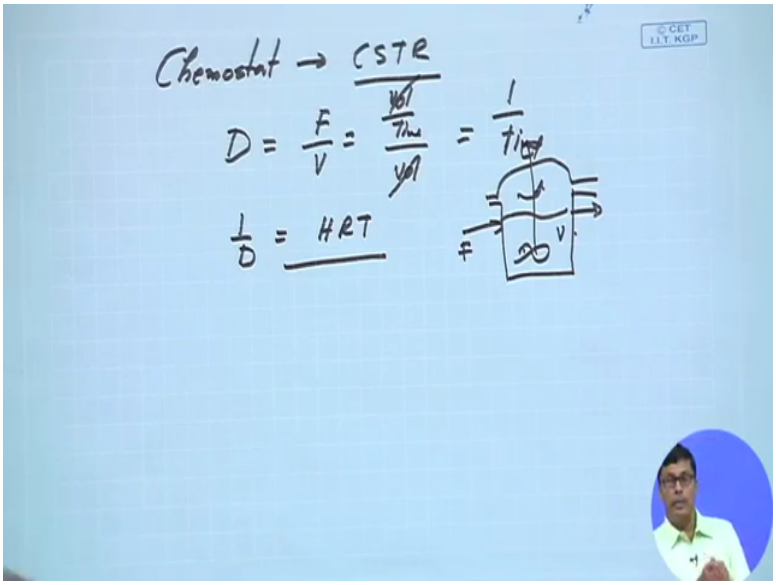




We considered as a chemostat use of continuous cell bacterial will get in a desired phase that is the we call it chemostat now assumption is that phase (())(24:20) feed excaudate is zero is feed completely mix and aerated. Liquid volume V steady state this control the pH we control the pH dissolved Oxygen and temperature and then the dilution rate what is the dilution rate I told you before also.

(Refer Slide Time: 24:40)



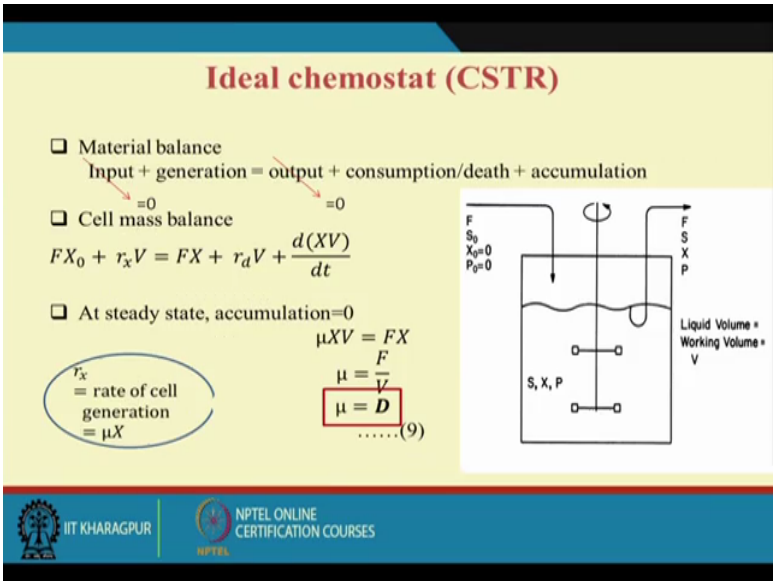


Dilution rate equal to F by V what is the F what is the unit of F volume per unit time am I right? And what is the V volume this is volume the following volume will cancel the unit is 1 by time the unit time inverse. The D unit is time inverse and one by D is nothing but what you called hydraulic retention time. Hydraulic retention time means how long a particular liquid suppose this is a starting reacted if I pass the liquid this is at the flow rate F.

And volume is how long this liquid particular liquid resize in the reactor and why you are interest for that because because that time it resize in the reactor that is the time of reaction that is why we are the more hydraulic retention time that that means the more reaction less

hydraulic retention time late less time we will get for the reaction here the for the growth of the cells.

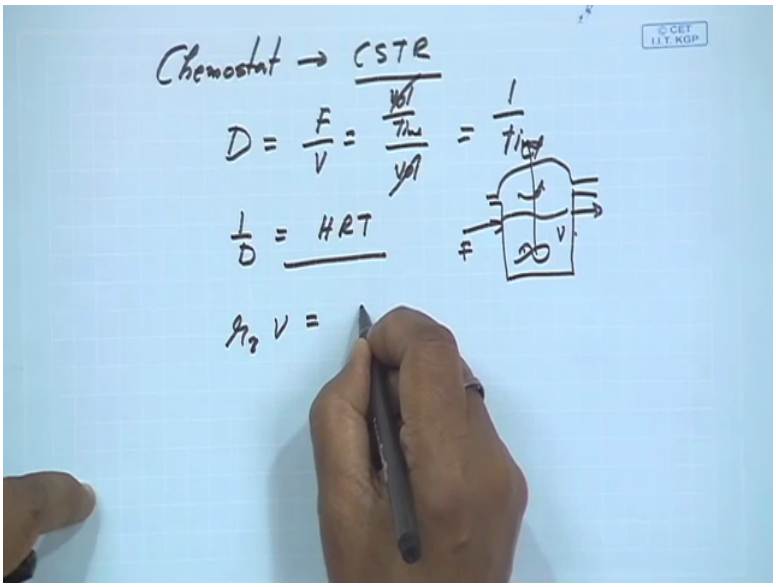
(Refer Slide Time: 25:52)

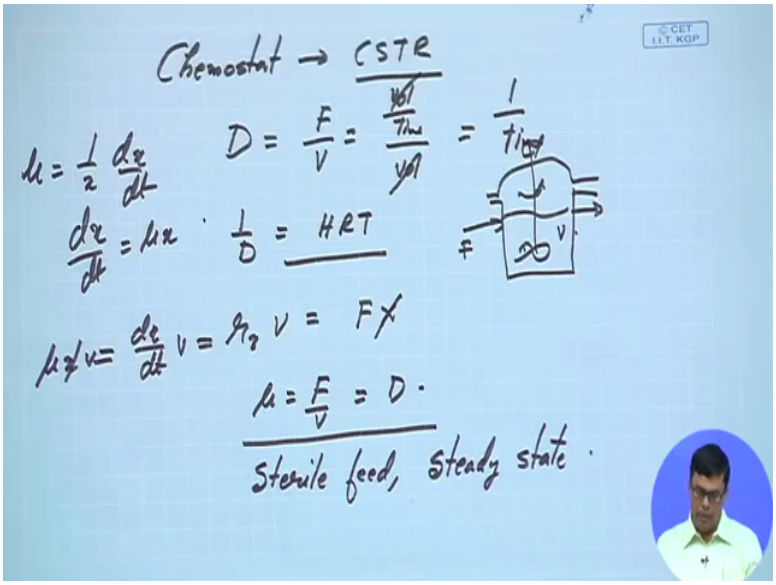


Now here how how we can analyse the chemostat process the that in case of cell balance we have this is the this is the input and this is the output of the cells so this is the input we can write F into F zero and then this is the generation of the cell then what is the output F into X and what is the this is the rate of death of the cell and this is a rate of accumulation now under steady state condition rate of accumulation is equal to zero.

So this equal to zero and also if we assume the rate of death of the cell is zero then also we assume this sterile feed sterile feed means there is no cell present in the incoming liquid incoming media then what will happen this will be equal to this this will be equal to this so if you if you multiplied by this then this is the thids DAI can I can write here so that everything will be cleared.

(Refer Slide Time: 26:58)

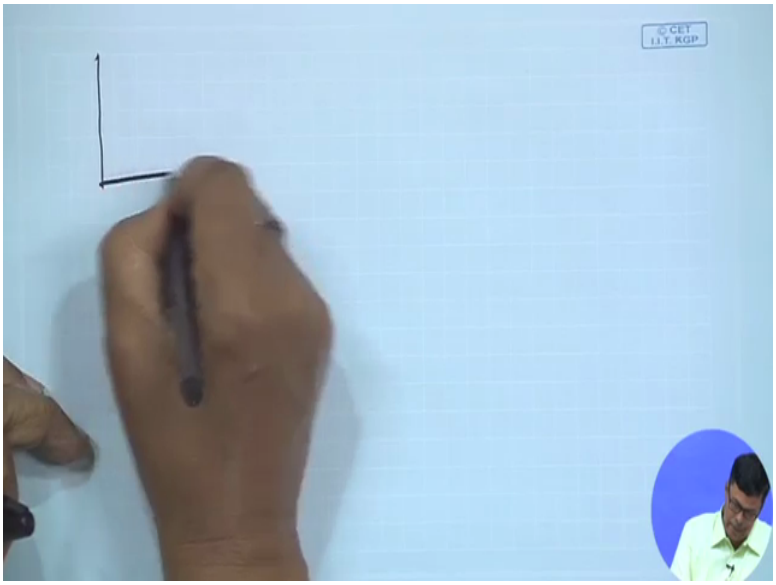


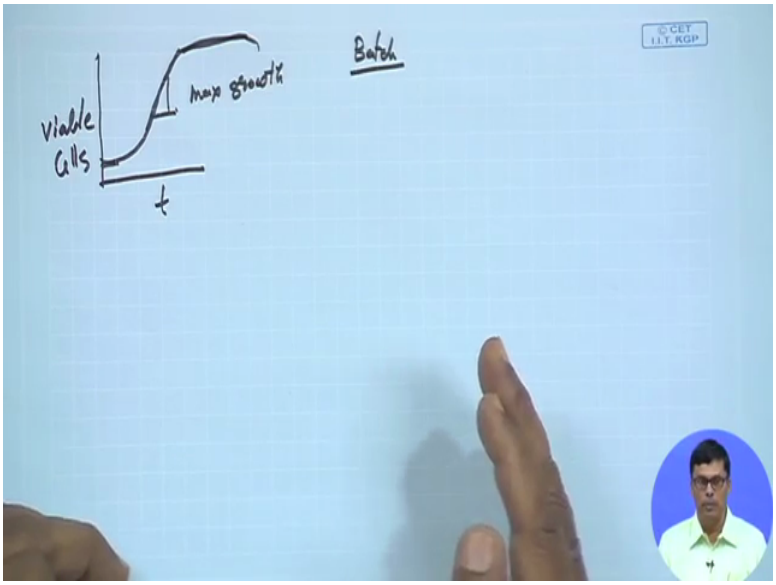


RX into V equal to F into X what is RX? RX equal to DX by DT am I right into V now we know mu equal to 1 by X DX by DT so I can write DX by DT equal to mu into X so we can we can what we can write here DX by DT equal to mu into X so this is equal to mu into X am I right so here X X will cancel.

So I can write mu equal to F this is V F by V that is equal to dilution rate so here this is this is that means mu equal to D this is a very important thing this is possible in case of sterile feed. Sterile feed and under steady state condition under steady state condition so this is the (()) (28:09) for getting this mu equal to DT. Now why it is required that let me tell you that here.

(Refer Slide Time: 28:21)

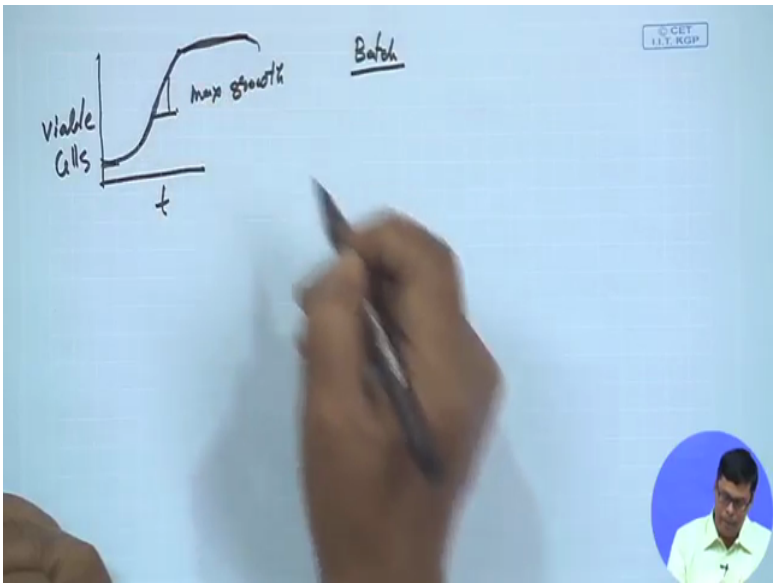




That if you look at the life cycle of the cell this is viable cell and this is time we have this the rate of growth maximum is this this is the log phase maximum maximum growth now now major drawback of the batch process what is the major drawback of the batch process? Is that you cannot hold the different phase this is a lag Phase log Phase and stationary phase you cannot hold the phase for longer period of time.

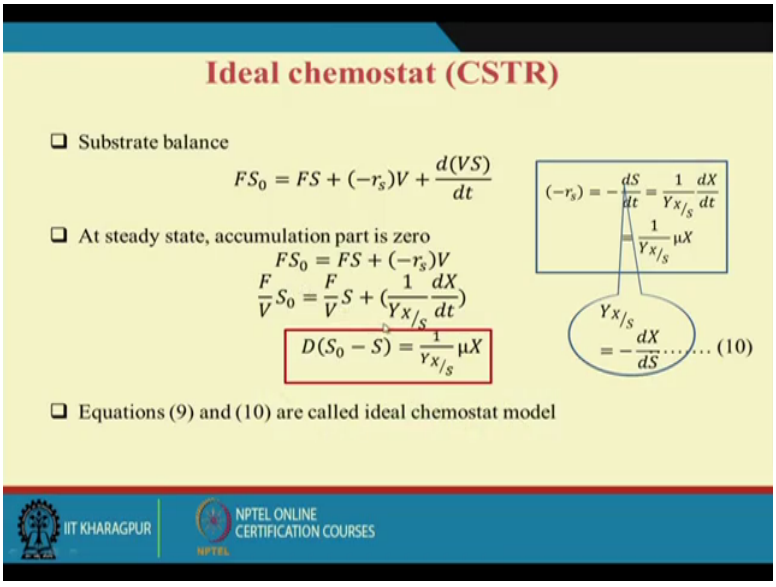
So as the time passes on one phase will be switching over to the other phase that is the major drawback of the batch process now in case of continuous system.

(Refer Slide Time: 29:11)



So you suppose we have maximum you want to produce Baker's yeast then the Bakers yeast production we are looking for high amount of the cell mass production so we are actually interested to grow the cell in the in the log Phase so we have just we have found that mu equal to under steady state condition sterile feed mu equal to D. The simple by controlling dilution rate it is possible to mention the log phase for infinite period of time and if you can do that we can have the maximum amount of cell mass production.

(Refer Slide Time: 29:46)

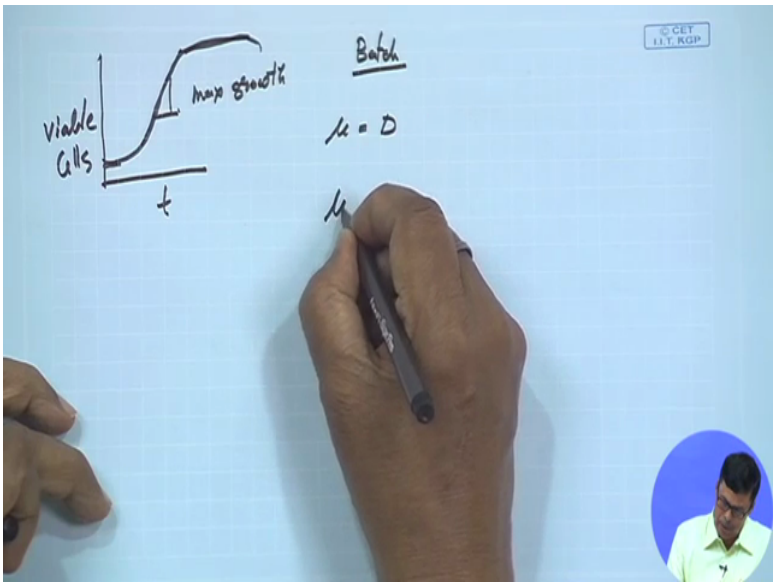


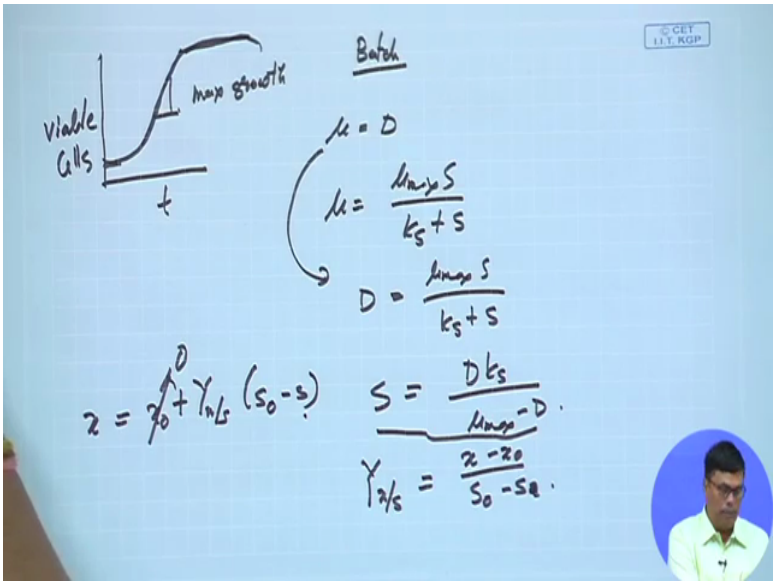
Now here we we try to do the material analysis substrate balance here this rate of substrate input rate of output this is a rate of substrate diffuse disappearance this is rate of substrate

accumulation but under steady state condition this will be equal to zero then we can come across this equation D into S zero minus S equal to this.

We can easily calculate and then we can apply the monod equation and then mu will be substituted by this and this called monod chemostat model this is this is called this is this is called monod equation and this is called monod chemostat model now rearranging this equation if you here let me let me tell you.

(Refer Slide Time: 30:33)



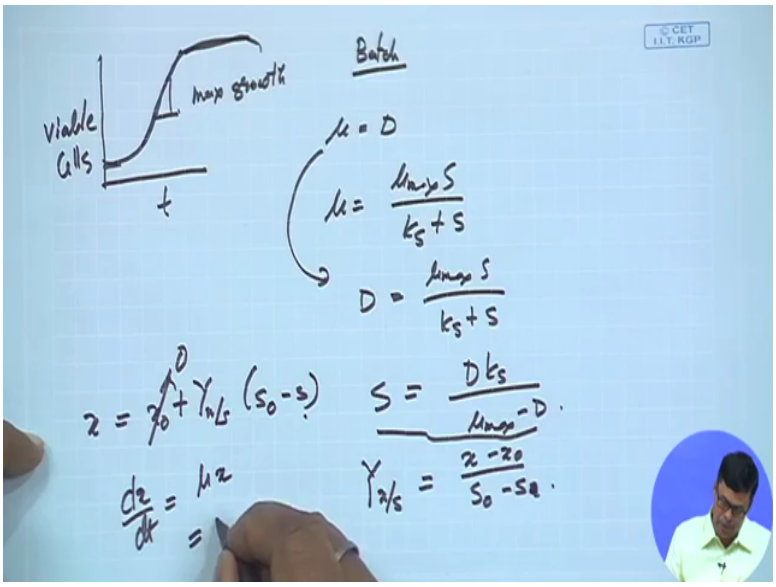


That mu equal to mu Max is KS plus S now under steady state conditions (())(30:43) mu equal to D so I can write D equal to mu Max S KS plus S so S you can easily calculate S you

can how you can analyse this is equal to KS mu Max minus D now why X by S equal to what a Y by X by S equal to X minus S zero divide by S minus S zero am I right now.

So I can write X equal to X zero plus Y X by S into S zero this is not zero this is S this is S zero minus S so this is sterile feed this will be equal to zero so if we calculate the value of this we can put it here we get the value of X now we can put the value we can get the value of X now here what is the cell productivity we get.

(Refer Slide Time: 31:41)



Cell productivity is the what if you if you multiplied what is the cell mass productivity is the DX by DT. Dx by Dt equal to what mu into X and mu under steady state conditions sterile feed mu equal to equal to D. D into X so this is considered as the that cell productivity this is like this so and this can be calculate like the to obtain the now I think this I should discuss in the next part of my lecture thank you very much!