Aspects of Biochemical Engineering
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Lecture - 33
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – III

Welcome back to my course aspects of biochemical engineering. In the last lecture I try to discuss the kinetics of substrate utilization product formation and biomass production of microbial cells and the process that we considered that is the batch process and also we discuss the monod equation, and monod equation is considered as the unstructured and unsegregated models and I try to explain what do you mean by structured model what do you mean by segregated models and; obviously, that unstructured and unsegregated model is considered as the ideal model it is not a real model.

Real model will be structured and segregated model and then we discuss about that how you monitored the concentration of the cells.

And I told you two type of cells we have one is called unicellular cell another is what we call filamentous cells or multicellular cells. In case of unicellular cell number is proportional to mass. The concentration of the cells can be expressed both either by mass per unit volume or number of cells per unit volume in case of unicellular cell. Now in case of in case of fungi or multicellular cell or filamentous cells always we shall have to express the concentration of cell which is used for explaining the in enzymatic reaction kinetics, and we discuss the what are the limitations of this equation that you know I told you that mu is finite when s is finite, mu tends to mu max when s tends to infinity, but it does not explain what will happen to mu when s tends to 0.

And in the addition to that there are two other limitations of this Michaelis Menten equation that it does not discuss the death of the cells because we know any kind of living population always there will be growing of the cells and death of the cells.

So, death of the cells is not as considered in case of Monod equation, and in addition to that there is substrate innovation and product innovation as per microbial system is concern that also we do not consider. And then we discuss about the life cycle of the cells, I told you the life cycle plays very important role because the reason is that in

process we have come across in to the lack phase, log phase, stationary phase and the death phase.

So, every phase has a significance as for example, lack phase we considered as a acclimatization phase, log phase is the active phase and stationary phase is a stagnation phase. And in case of dead phase that is done with a organisms are dying phase.

So, you know that so, why it is important the reason is that whenever we prepared any kind of inoculum the or you know culture for any kind of permutation process we shall have to inoculate in between the mid log phase to lid log phase that is the culture we should prepared.

Now, in addition that to that we discussed other different type of growth models that we recommended by different type of scientist, and also we discussed about the substrate inhibition and product inhibition and also we taking pyrite model and port model.

Now, today I am going to discuss that how this in the CSTR system or when CSTR means continuous start time reactor; when continuous start time reactor we used in the biological system, we call it chemostat process. So, how in the chemostat process we can we can use the living cells ? So, do that lecture will be mostly concentrated on that.

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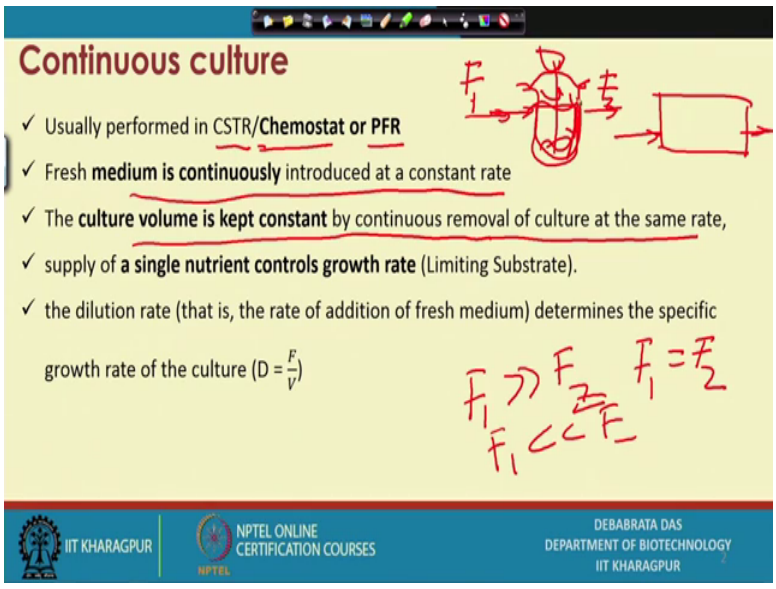

Now, if you look at that this is a continuous process we have already I already told you that we have two type of process we have one is your CSTR, what we call chemostat

another is the plug flow reactor. Now chemostat basically this is simple they reactor this is like this and so, there is a continuous inflow and outflow. And plug flow reactor it is like this that you know there is a tube tubular reactor and equate coming one end and product is going out both are continuous reactor. Now here we have we have uniformity in the reaction mixture that by using the mechanical stator here there is no stator.

Not only that during the movement there should not be any axial mixing radial mixing is permissible not axial mixing. Now place media is continuously introduced into the at a constant rate. So, we take it a phase media here, and culture volume is kept constant in the by continuous removal of the culture now suppose F is the flow rate, now and we here also we select to maintain the flow rate F.

Now F suppose this is F and this is the F 2 and if F 1 is more than F 2 then what will happen that your volume of the reactor that keep on increasing that is undesirable and if F 1 is less than F 2 then volume of the liquid that will keep on decreasing with respect to time.

So, this is the problem; now when F 1 equal to F 2 then in all in the volume of the reactor will remain constant am I right. So, this is the this is the significance of this particular thing, then we have a single nutrient control growth we can what you call now I told you the media comprises of n number of component we have carbon source, we have nitrogen source we have minerals and so, we have vitamins am I right.

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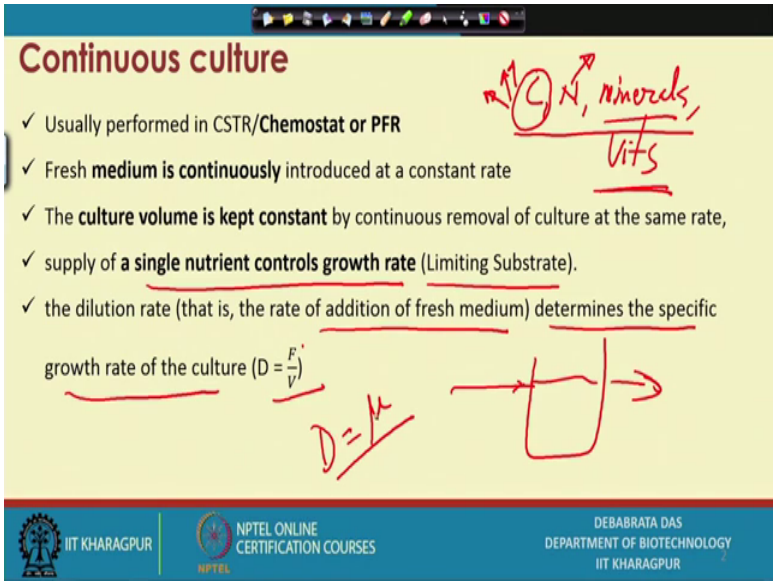

So, you know that everything has some contribution to the living system; carbon has three contribution it goes for the cell mass formation it goes for the source of energy and it goes for the product formation N nitrogen mostly it is used for the growth of the cell, minerals and vitamin they mostly contribute as a cofactor in the in the metabolic pathway or if the enzymes involved in the metabolic pathway.

Now, so if you want to study that what is the effect of this component individually we can easily study with the help of continuous systems. Just we can change the concentration here, here we change the concentration immediately we can find the changes here. The dilution rate that is the rate of addition of fresh media determined by the specific growth rate.

That is D equal to F by V F is the flow rate and V is the; because there are D is equal to mu I shall show you in the next slide a dilution rate because the because here I want to point out one thing that when we study our life cycle this is the x this is the cell mass concentration, and this is time.

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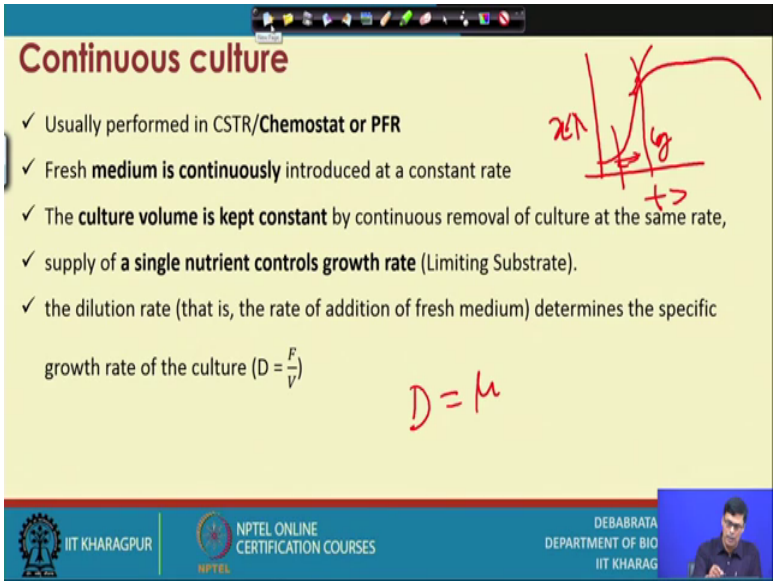

So, we have this kind of that you know life cycle we have. Now suppose in case of beakers is fermentation process we are interested to operate it in the in the log this is the log phase am I right because log phase we will get the maximum rate of cell mass formation.

So, that we can maintain by controlling the dilution rate by D equal to mu that next slide I hope you will get that information.

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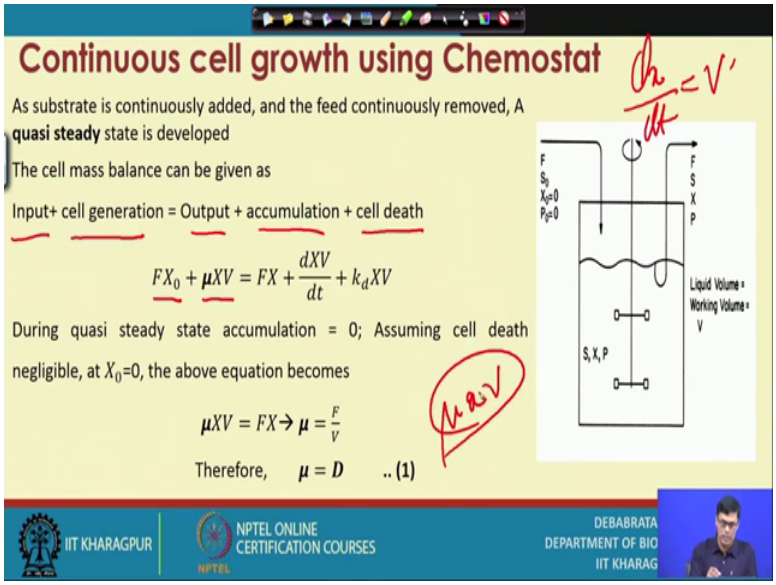

Now the continuous growth cell growth using the chemostat as substrate continuously added and feed continuous remove, a quasi steady state is a developed. Now what I want to want to mean here that in the chemostat process that quasi steady state conditions means it is not exactly steady state, it attains to steady state. Now suppose I told you can remember whenever we operate any kind of continuous process first we operate in a batch mode.

And when your rate of cell mass growth is maximum then we start feeding the media continuously and take out the that are you know fermented media continuously. So, this is like this, F is the flow rate S 0 is the initial substrate concentration, x 0 equal to 0 when you have sterile feed sterile feed means there is no cell present in the media and P 0 means no product present in the media. So, here you will get this as the F flow rate is the substrate concentration X and P. Now this we can have under steady state condition and steady state condition see two steady state condition, is possible when and only when we operate the system for infinite period of time.

Now how you can do the cell balance? Now we have the we have the equation that a rate of input a plus cell generation equal to rate of output accumulation of the cell and cell death. Now here under steady state condition that F into X X 0 is the cell that is the input

in the system cell generation is that this is equal to what I can write d x by d t into V am I right? Now d X by d t equal to mu into x. So, I can write this is mu into X so, this is exactly what we have written here.

That mu x into b and this is the output F into X because under steady state condition X is the series to substrate concentration, and these are the rate of cell that is the accumulation of the cell now this will be equal to 0 in under steady state condition, and this is the this is the rate of death of the cells.

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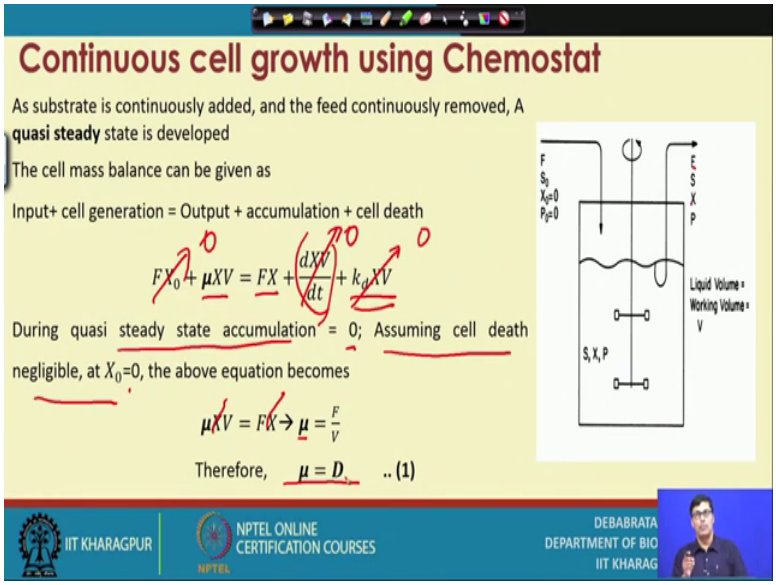

So, quasi steady state conditions the rate of accumulation that it should be equal to 0 assuming the death of the cells is negligible, and X 0 equal to 0, now this above equation I can write that this is so, this is equal to 0 and I assume this is also equal to 0. So, the what will happen? Mu X V equal to F into X . So, X X will cancel am I right.

So, we can write this is mu equal to F by V; means mu equal to D. So, what I was telling in the last previously that by simple by controlling dilution rate, it is possible to maintain a particular phase of growth for infinite period of time.

So, this is very that is that is the advantage of this per chemostat process. Now in compared to the batch process we can we can referred that, in the batch process this was the major drawback why? In the batch process you cannot hold a particular phase of growth for infinite period of time, but in the continuous system you can operate a

particular phase of growth for infinite period of time so, you can get maximum amount of cell mass formation.

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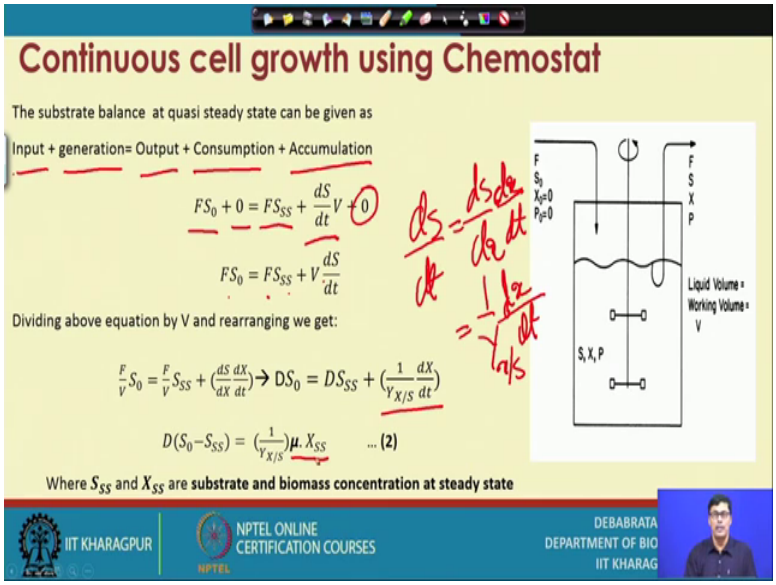

Now, if you if you do the substrate balance, the previously it was cell mass balance; now if you do the substrate balance then again we can write rate of substrate input plus rate of substrate generation, rate of substrate output consumption, and accumulation. So, we can write F into S 0 a substrate generation means the substrate cannot be generated in that the reactor show these we can assume to be 0, this is the substrate output this is the rate of formation it is a degradation of the substrate this will be plus, and under steady state condition this will be equal to 0. So, we can write F into S 0 into F SS equal to V into d S. Now d s d s by d t what we can write? D s by d t we can write d s by d x into this is equal to d x by d t am I right we can write like this.

Now d x by d s we can write this is 1 by x by s into d x by d t this is this is what? What is written here and then this is this has what has come, this is 1 by n d x by d t equal to what? This is mu into X s s what is this X s s stand for steady states cell mass concentration X s s stands for the X s s is the steady state cell mass concentration. So, this equation we can write in this form D into S 0 minus S s equal to 1 by Y x s s mu into x 0.

So, this is this is how we can we can we can we can develop the correlation between the steady state substrate concentration and the cell mass strain stress cell mass concentration.

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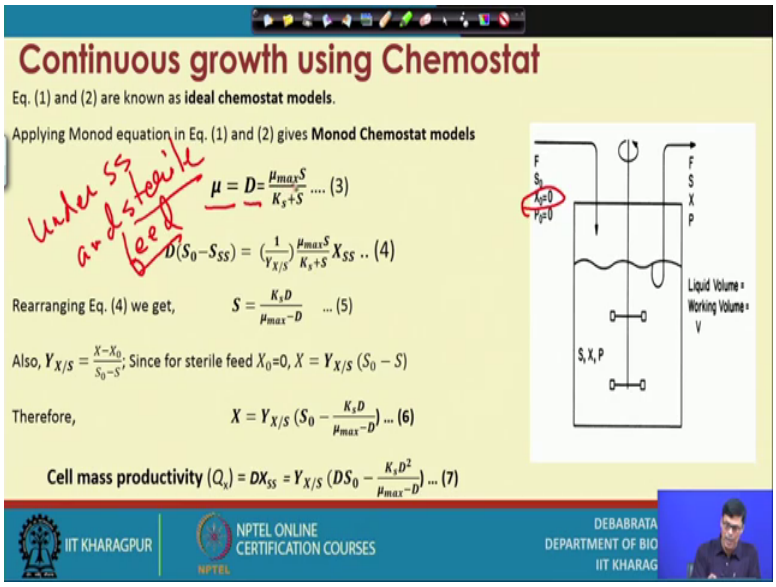

Now, next is a very important thing is that how this dilution rate affect the steady state condition. So, this let me explain that.

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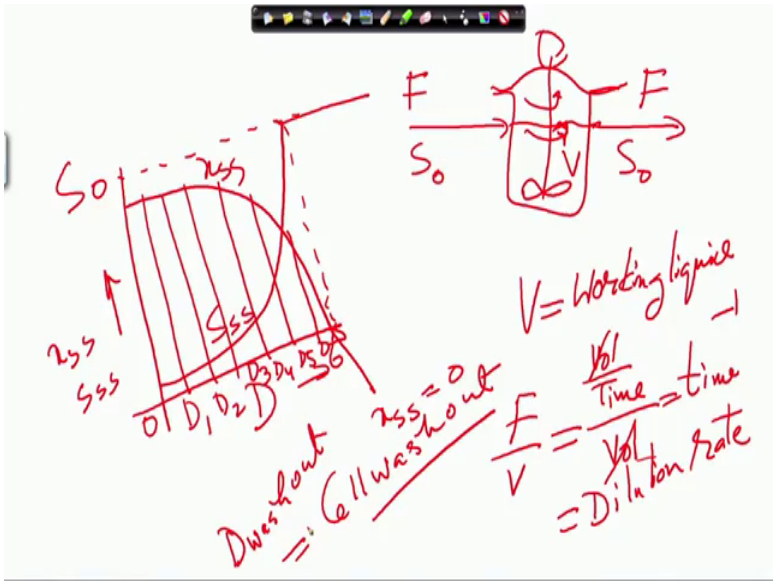

Now, let us assume this is this is we have this is the start time reactor am I right this is the this is start time reactor and the so, the substrate is coming this way and product is going this way.

So, here F is the flow rate and this liquid volume we can consider as V. V is called the liquid volume what we call walking volume because we assume that reaction take place only in the liquid phase. So, now, it does not take place in the free phase. So, this is called walking volume. So, this is also F so, what we can we can do we can I told you F by v what is the F by v what is the unit? This is volume per unit time am I right. And this is what this is volume the this volume will cancel.

And this will be time inverse; this is this is equal to dilution rate this is equal to dilution rate. So, this is dilution rate you can you can control you can change the dilution rate as you because V is constant. So, if you want to a increase the dilution rate, you just increase the flow rate. Volumetric flow rate of the liquid you can change that dilution rate. Now if you see the correlation between D versus the x s s and S s s what is the x s s I told you? This is the steady state cell mass concentration and the S s s is the steady state substrate concentration.

Now, this is steady state cell mass concentration and this is actually the steady states substrate concentration. This is the S s s and this is the x s s. So, now, that here you can see that at different dilution rate this is D 1 this side is 0 this is D 2 this is D 3 this is D 4 this is D 5 this is D 6, but here what is happening? Here there is no cell present in the reactor, x s s is equal to 0 am I right? The x s s equal to 0 what you call we consider this as a cell washout what do you mean may washout of the cell? Because there is no cell present in the reactor because the total reactor is free from the cells now if there is no cell present in the reactor.

We what will happen the S 0 that S 0 here and here also will be S 0 because no cell no reaction take place. So, this is exactly this point will be S 0 so, this is a corresponding point is the is the this is called D washout and D washout there is no cell present in the reactor. Now this is the major problem that we have in case of sees that chemostat process. Now question the question may be raised that why this happens. This happens due to the say two different reasons one I can explain that you know we know the generation time what is generation time?

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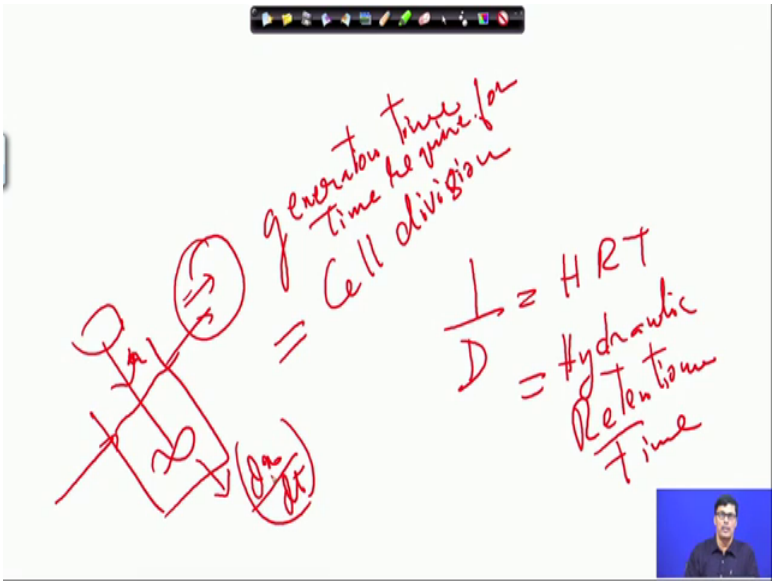

Generation time is the time required for cell division, time required for cell this is the time required am I right for cell division. So, you know the suppose you are passing the cell in the what is 1 by D? 1 by D is called HRT. What is HRT? HRT is called hydraulic retention time am I right. So, how what is hydraulic retention time? That means, how long you allow your liquid to recited in the reactor, how long that you know and.

Why it is important? Because that is the time of reaction because the time you are having the liquid resides in the given that is the time of reaction. Now if your generation time and hydraulic retention time is less than that the generation time what will happen? Before cell multiplies you are taking off the cell from the reactor.

So, there is no self reason in the reactor another way it can be explained there suppose this is a continuous reactor and you are passing one end like this. So, if suppose that here rate of growth of the cell d x by d t and rate of cell mass that is going out from this system that is that if .

If a rate of cell that is going out the system is more then rate of growth of the cell then what will happen? The cell mass concentration in the reactor keep on decreasing and a time will come when there is no cell present in the reactor am I right. So, let us see that here what we have written this is that we have already seen this is mu equal to D; when mu equal to D under steady state conditions and the sterile feed what is sterile feed means here x 0 equal to 0 the input there should not be any cell present in the in the feed

that is why we call this sterile feed then the then are only m equal to D this is equal to this is Monod equation we know mu max S case.

Now, previously we have done this that you know the substrate balance and in the substrate balance we have this equation. In this equation if we if we put the value of mu this is mu max is k s plus S then we considered this as a Chemostat model this considered as a this is called Monod chemostat model. Now our Monod chemostat model is how it is done? Because the we when we have the this substrate balance or cell mass balance and in the substrate balance and cell mass balance and when we put the Monod equation, then we considered that a Monod chemostat model the same thing we can we can be walk out in case of cell mass balance.

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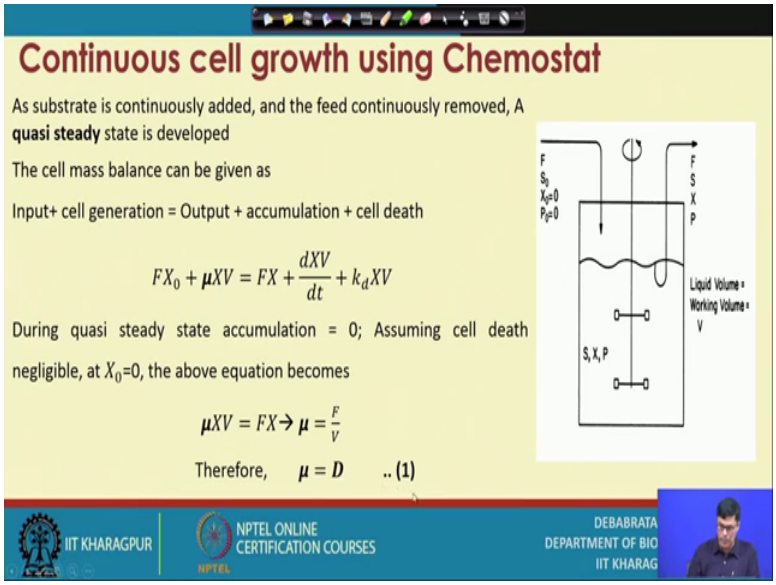

Also we can we can we can we can find out that in cell mass balance also we can write the Monod chemostat model.

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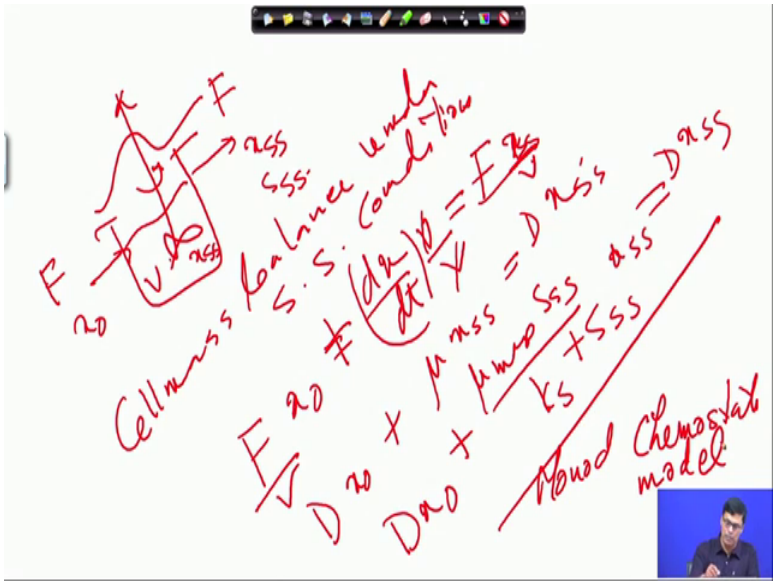

Now, let me show you how you can do that suppose this is a reactor and this is the input and there is the output now we have this reactor, this is F this is x 0, this is the F into x s s and this is this is also x s s, and this is S s s, am I right this is v. So, if you write the cell mass balance under steady state condition; under steady state condition what will happen that, the rate of accumulation equal to 0 rate of input is the F into x 0.

Am I right what is the rate of generation of the cells that will be d x by d t into v. And what is the rate of output F into x 0 x not x 0 the F into x this is the rate of that and then we can write this is rate of accumulation; obviously, that will be equal to 0.

Now if you divide this both the side by v this is v is there we can divide by v then what will have this v the f by v equal to d D into x 0 plus what is this? Mu into x s s what is this? v v will cancel and this is will be D into x am I right? Now this is equal to x into x 0 plus mu equal to mu max S s case plus S s s into x s s am I right equal to this is s s, x s s this is equal to D into x s s. Now this equation also we call it Monod Chemostat model. So, we have we have two Monod Chemostat model; one is with respect to we with respect to with respect to substrate balance another is with respect to cell mass balance.

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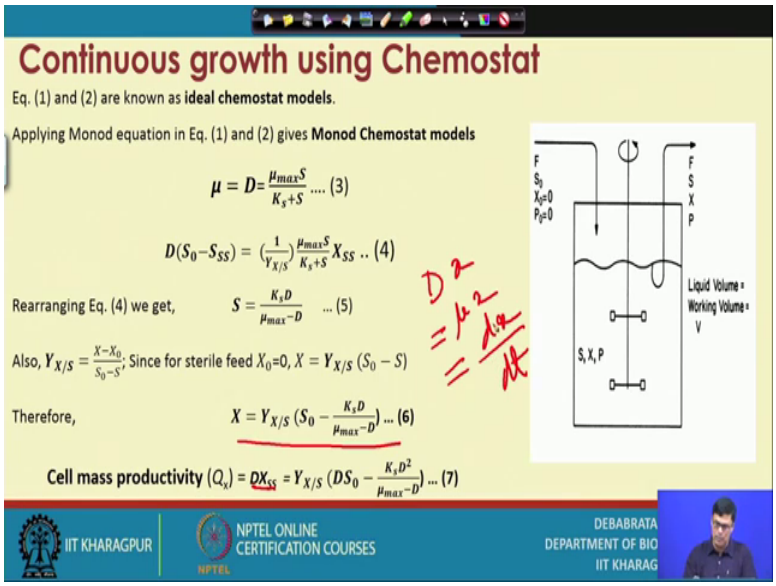

Now here the interesting thing is that that the steady state substrate concentration as (Refer Time: 23:38) to the cell mass concentration we can easily find out. Now we have this equation am I right.

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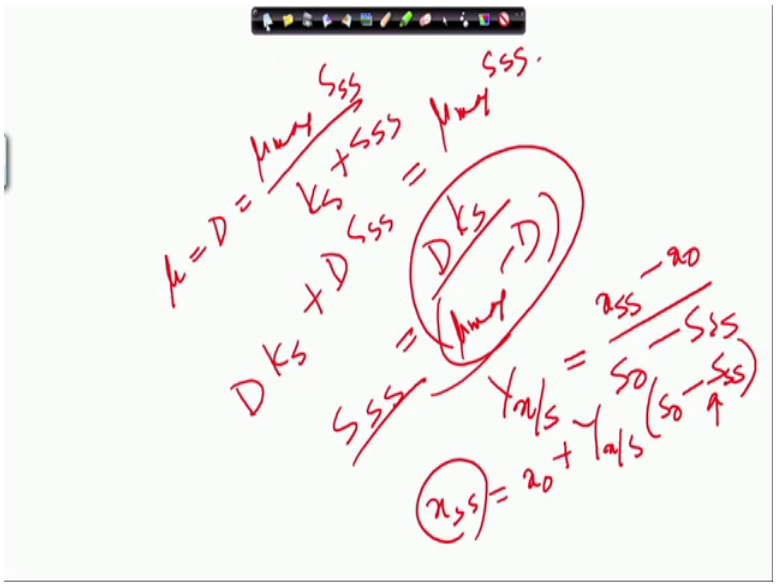

Now if you look at this equation that is mu equal to D equal to mu max S s s k s plus S s s am I right. So, this is equal to I can write D k s plus D S s s equal to mu max into S s s . So, I can So, what we can write? S s s equal to D k s equal to mu max minus D so, we

can easily find out the steady state substrate concentration. The once we find out steady state substrate concentration we can find out x value.

How we can find out x value? Y we know, Y x by s equal to x s s minus x 0 this is equal to divided by s by s S s s am I right . So, what we can write this is x s s is equal to x 0 plus Y x by s into S 0 minus S s s. So, if we put the value of S s s here what we have done here we can find out x s s value, this we can easily do that. So, this is the exactly what we have done here you see that you know this is the x equal to a x equal to this. So, we can easily find out then if we multiplied by a D there is dilution rate, then what it D into x? This is D into X s s equal to productivity because there are already D into x equal to we can write mu into x and this is equal to d x by d t. And this is d x by d t means (Refer Time: 25:29) of cell produced per unit time this is called productivity. So, the this we can write in this form.

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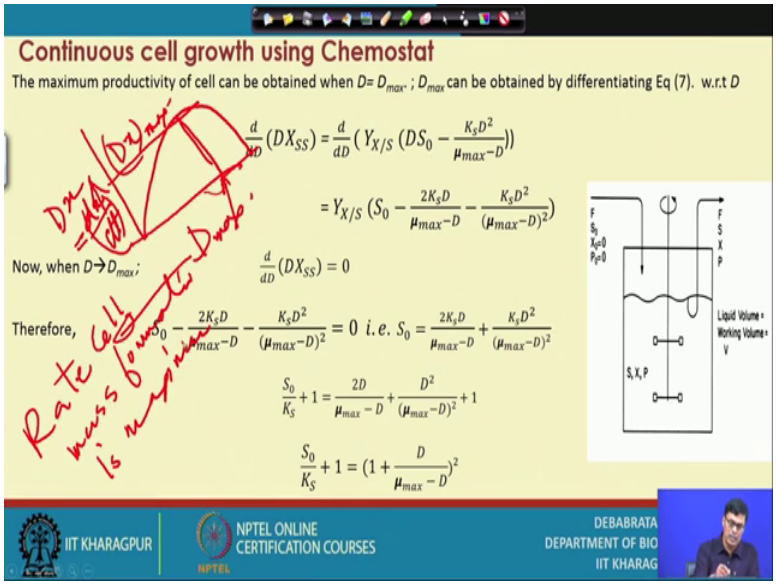

Now, when you when you there is a very interesting thing when you plot that D versus D versus D into x; D into x means d x by d t am I right rate of cell mass formation and this what is the plot? Plot is like this. Now in this plot here what is this? This is equal to D x this is maximum am I right. So, what is D x maximum then with this point we call it D max. So, what is D max? D max means when the dilution rate at which the rate of rate of cell mass growth cell mass formation is maximum.

So, this is this is what we can we can we can find out now here in this plot.

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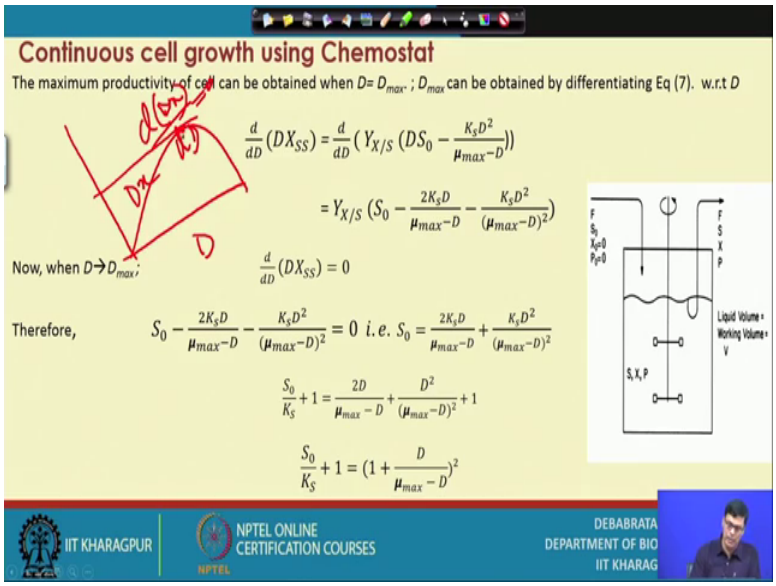

If you look at like this so, this is D x am I right this is into D x and then D. So, here I can write d d x by d d this should be equal to 0 this is play to here am I right. So, this is exactly we if you look at here.

Here we have we have this D x equal to Y x by s DS 0 this. Now if we differentiate this with respect to with respect to d d D we if we do that then we will be coming across this equation we will come across this equation and for y the DX this is d and d DS 0 this is the equation and if you differentiate D then it will be 1 this is S 0 and if we D d square and this two term is there. So, this will be equal to this value and then we put this that d at when mu D is transferred D equal to in 2 D max d d by DX that will be equal to 0 then if you put these conditions, we will we will you solve this and we find this equation this equation we find and finally we will come across this equation mu equal to mu equal to mu max is KS by apply this.

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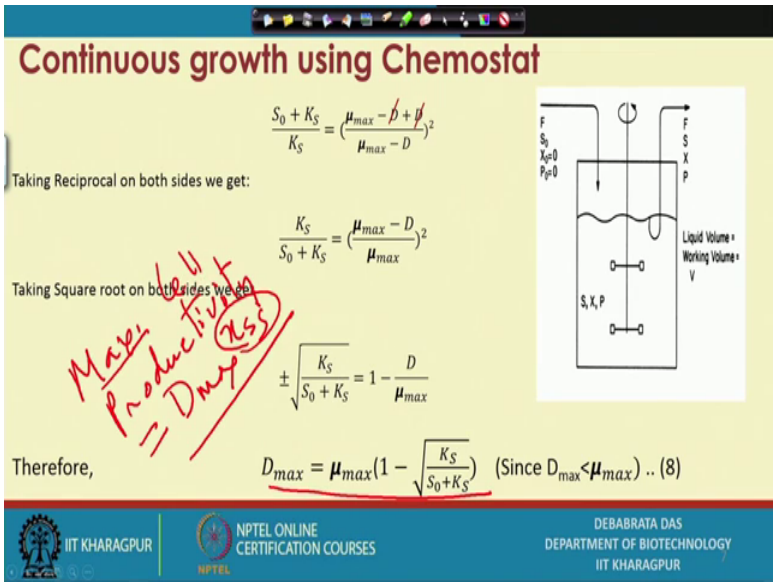

So, this is the what is So, this is the what is this D max? D max is the dilution rate when you will get the maximum amount of rate of cell mass production. Now what is the maximum productivities? Now I if I ask you what is maximum productivity; now the productivity is usually expressed mass per unit volume; maximum productivity means when the rate of cell mass formation is maximum.

So, at D max your rate of growth of the cell is maximum, now at this at that particular situation that whatever cell mass concentration is there. So, but we have D max and x s s, at that condition if you multiplied that corresponding a steady state cell mass concentration, then we will get the maximum cell mass productivity . So, maximum cell productivity we can easily calculate.

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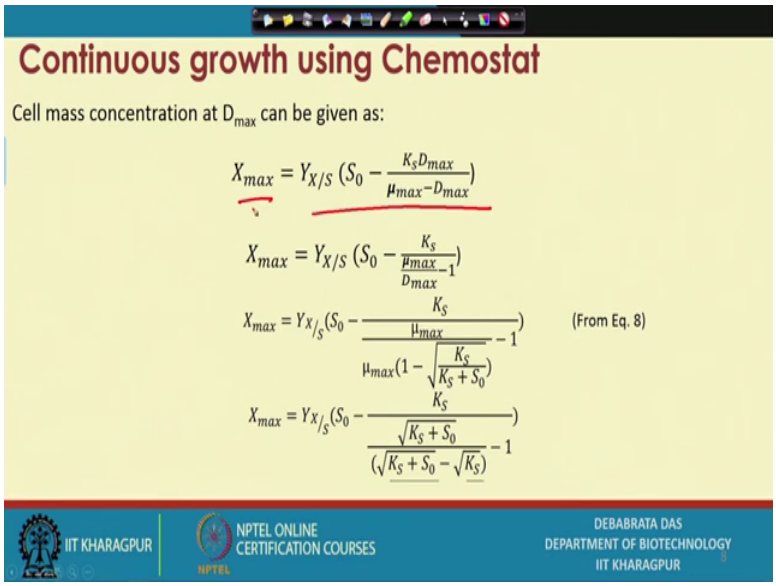

So, this is exactly and from that we can find out the maximum cell mass concentration also x. Since the this is the D max that is the So, we can easily find out that maximum cell mass concentration and this is how we can solve in this form.

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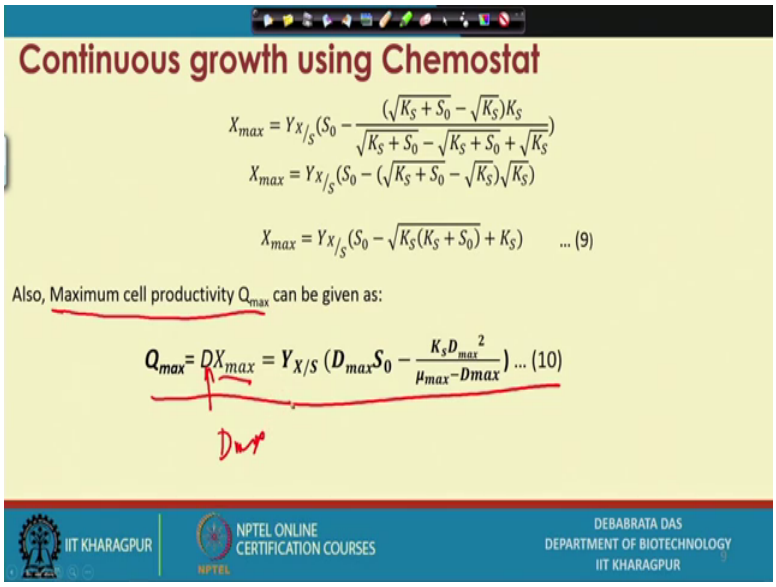

Final form will be coming this, I told you this is a d into x max that you know this is and this is we considered as a D max if you have D max we have x max and if you multiply that and this equation correspond to the maximum; maximum cell mass productivity the maximum cell productivity can be calculated with the help of this equation.

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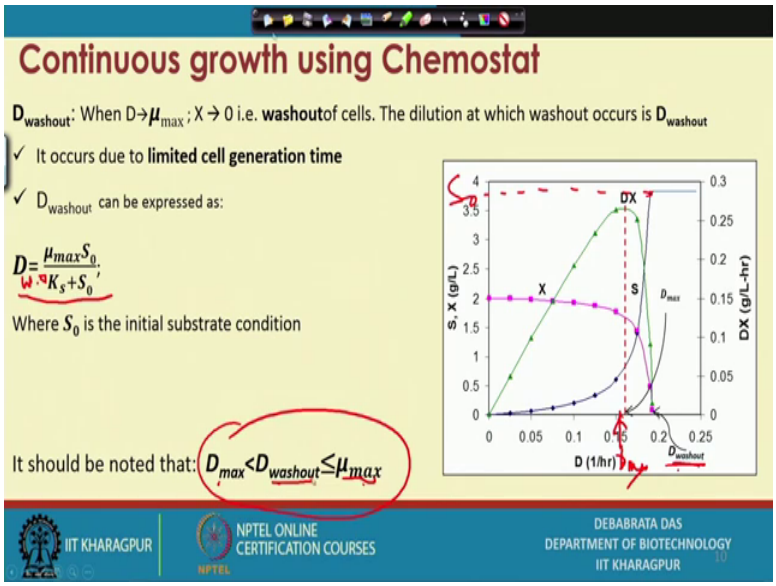

Now, that here we give you some graph, but what I have shown you, that this is the D x, this is the X value and this is the substrate concentration am I right and this correspond to x 0 am I right.

So, what is the X 0? Where at D wash out that S value will be S 0, then what will be the d value at D washout value? D washout equal to d mu max into S 0 K s plus S 0. So, we have seen that this is equal to D max am I right? This is equal to D max and this is the D washout. Now when we want to produce more cell we are interest to operate the system at D max, but you know that industry how we operate the flow rate by controlling the bulb now if we increase the bulb little bit more.

Actually operated they do this work, but when we open a little bit more there is the every possibility that D max can meet the D washout situation . So, this is the major problem that we have with the biochemical industry that So, this problem can be overcome by two ways either you recycle the cell and odd you immobilize cell on the solid metric.

This is the true I shall come back now if you look at this that correlation between D max D washout and mu max usually the correlation is like this D max you can see, this is D max the D max will be less than d wash out am I right? But D washout usually equal to or less than mu max value; this is the this is the correlationship that we have.

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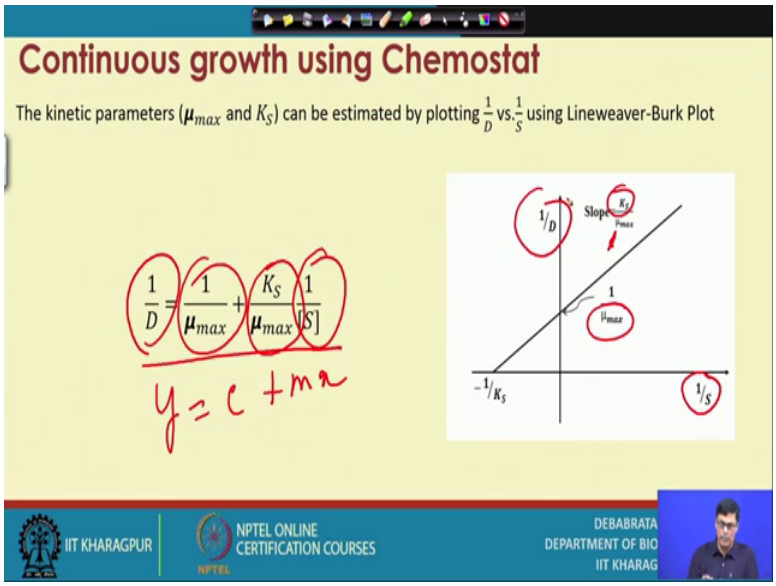

And if we want to now we have shown you already how in the batch process, we can find out the value of cell growth kinetics, now in case of chemostat also it is possible to find out the cell growth kinetics and if you write this equation in the form of Lineweaver Burk plot. Then what do you have to have do? We have to plot 1 by D versus 1 by D because the everything is constant what is this? This equal to y equal to C plus m x am I right.

So, if you plot 1 s one with you will be having straight line, the intercept will give you the value 1 by mu x, and this slope will give you the K S by mu x. So, whatever mu max value you have you put it here. So, we will get the value of K S. So, it is in the in the chemostat process also it is possible to find out the kinetic constant.

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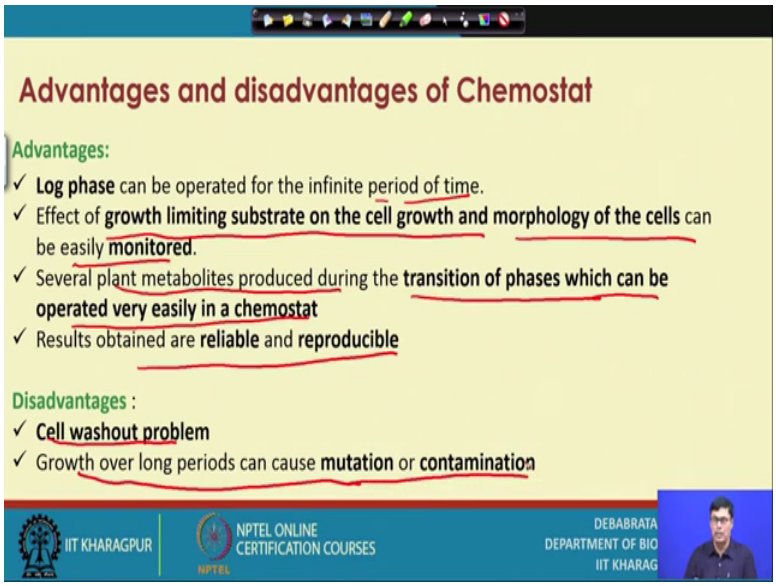

Now what is the advantages we have with the chemostat process? That log phase can be operated for infinite period of time, the effect of growth limiting substrate on the cell growth and morphology of the cell can be easily monitored because I told you that cell conferences compress the in number of components suppose we want to find out the effect of the individual component on the on the particular process that easily we can find it out.

Several planned metabolites is usually produced through the transition of phases, which can be easily operated in the chemostat. At simple by controlling dilution rate we can have 2 and 4 that movement of the phases. So, we can have more metabolite formation; and results obtained are reliable and reproducible. Major disadvantage of the chemostat is the cell washout problem, and growth over the long period we can caught mutation and contamination, this is the major problem of this process.

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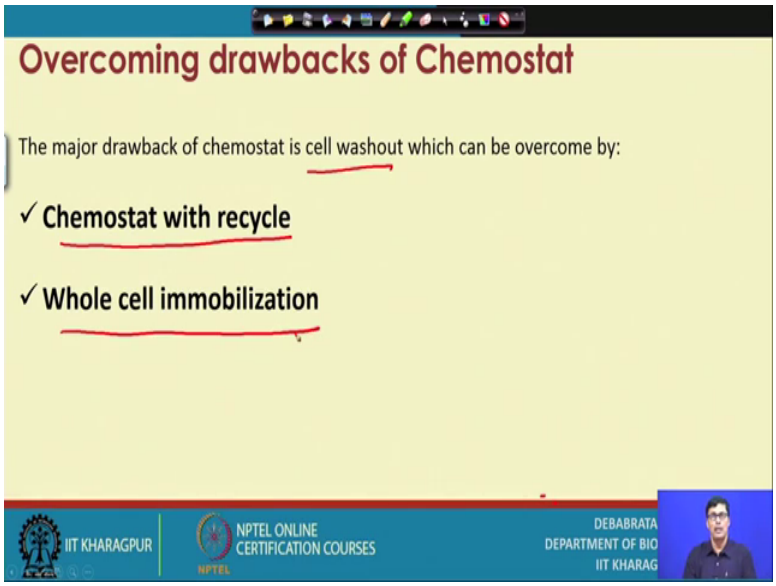

Now, as I told you that this drawback that you know cell washout that is the drawback can be overcome by two ways; one is called chemostat with cell mass recycling and wholesale immobilization system. This I will discuss in my couple of lectures.

Thank you very much.