Aspects of Biochemical Engineering
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Lecture – 36
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – VI

Welcome back to my course Aspects of Biochemical Engineering. Now, in couple of lectures we are dealing with the living cells and living cells both in the form of free cells as well as in the immobile cells. And we discussed different processes like, batch process, and continuous process. We have come across chemostat and the plug flow reactor.

Now, this particular lecture is something different, because we are going to discuss a new type of process, what you call fed batch process. And I mentioned before that for the substrate inhibition, we usually prefer the fed batch reactor. Because, in that and I here, I want to point out in case of product inhibition, we usually prefer the plug flow reactor.

So, now, question comes how the fed batch system can be operated, and how it can be analyzed. So, this I am going to discuss in this particular lecture. Now, first let me consider that you know that, what do you mean by fed batch process?

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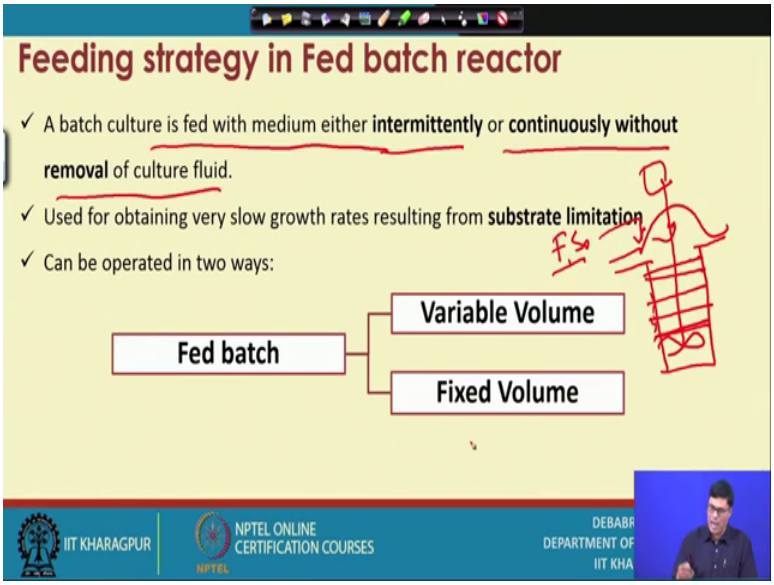

Fed batch process means, that the batch culture that is fed in the media, either intermittently or continuously without removal of culture fluid.

So, what does it mean? That you know suppose, this is the reactor, am I right? This is a reactor. So, we start with small volume. And then this is the reactor; this is a moving. So, there is a input. We may pass the as a particular flow rate of substrate, but slowly sell the liquid the height may be increases. And when it comes here, then we stop the flow. Then, they let the reaction complete then we take the liquid out.

So, this is the fed batch, we are and this feeding may be of 2 different types. One is Variable Volume, one is Fixed Volume. Variable volume is slowly had you have this you can increase like this, you can have the line. And fixed volume means with respect to time, we can keep on adding this some volume with within this particular reactor. So, these are the things that we have usually used for obtaining very slow growth rate resulting the substrate limitation, and can be operated 2 ways one is variable volume, another is fixed volume. Now, question comes how you ensured your substrate utilization is more. Let me, analyze that.

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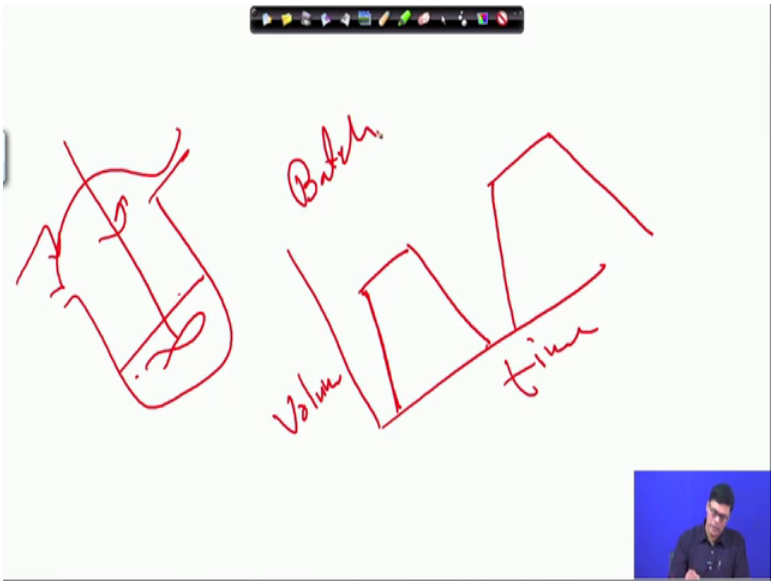

Suppose, what I am saying that this is the reactor. Now, this is moving like this. Now, when you consider the batch process, how you; how what is the basing the feeding strategy we have? Like this. We take the material allow it to react, and then take out the

material. Again, we have take the material then, allow it like this, this is with respect to time. This is volume, am I right? This is the batch process.

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Now, if you consider the fed batch process, what is the strategy that we have? We have here also we have volume, and this is time. So, we initially we start with some volume. I have shown you in the reactor, we start with some volume, this is the volume. Then, we increase slowly. And they attends the then another this volume. What is the final volume, then we take the material out, am I right? Then, again we start this operation again, we increase the volume you can take it out. So, it is that this is stepwise we can operate the system. Another way how we can operate? So, this is the way you can find out another way that; now, here, one interesting thing is that, if you look at the substrate concentration, substrate concentration with respect to time.

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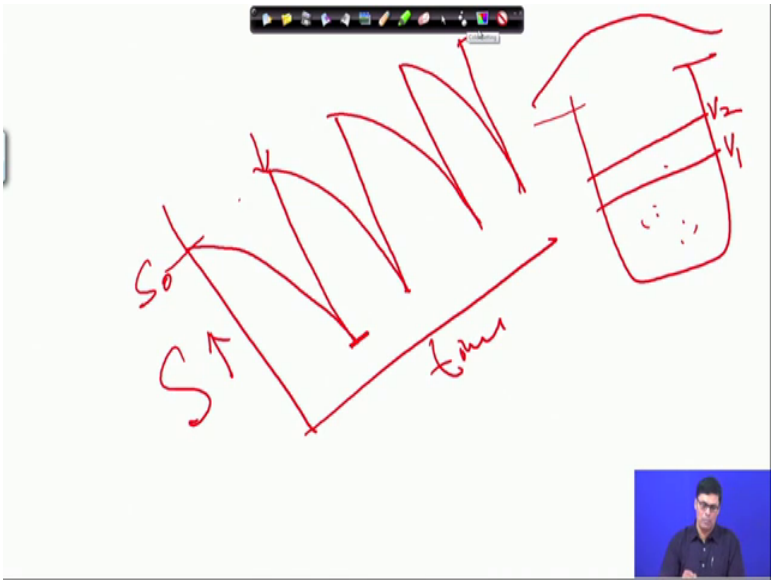

The substrate concentration will keep on decreasing, suppose in this reactor you put the your raw materials then, allow to react, then what will happen? That substrate concentration will decrease with respect to time. When it is comes to minimum, then again you feed it. So, this is you can increase the feed like this.

Now, here, you imagine here, you have with the volume v1 now, next you when you add this is v 2 now, when you add v 2 this will be little concentrated material, am I right? So, that it can you can have the substrate concentration what do you have is 0. The same level again, you can decrease like this, again you can increase like this, again you can decrease like this, again you can increase like this and until unless you reach the final volume.

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Now, if you look at the other type of strategy that we have like S versus time. So, what we can do we can we can first we start with the volume, let the reaction take place and then, we start feeding the that the substrate decreases then, again we feed after we come it here, coming here this is S 0 value, and then we continuous feed in the manner. So, that you know your this value remain is 0, and then finally, when release the final volume with. So, substrate concentration decreases to this. So, this different type of feeding strategy we can use, in case of immobilization system.

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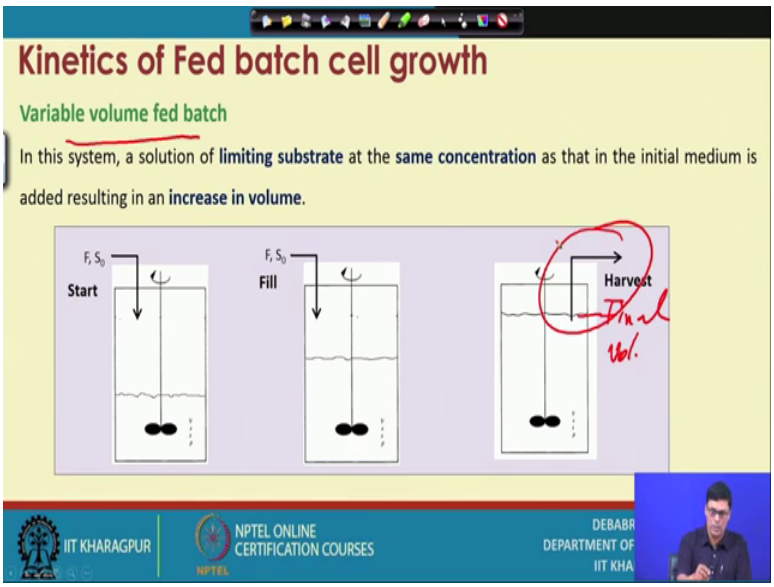

Now, here, we have given the example of the variable volume fed. So, we start with this volume because, this is the start then we increase little bit, you can see that below and final volume (Refer Time: 06:40) then, when there is the final volume this is the final volume, am I right? Final volume when is reaches the final volume then, we do the harvesting of the material.

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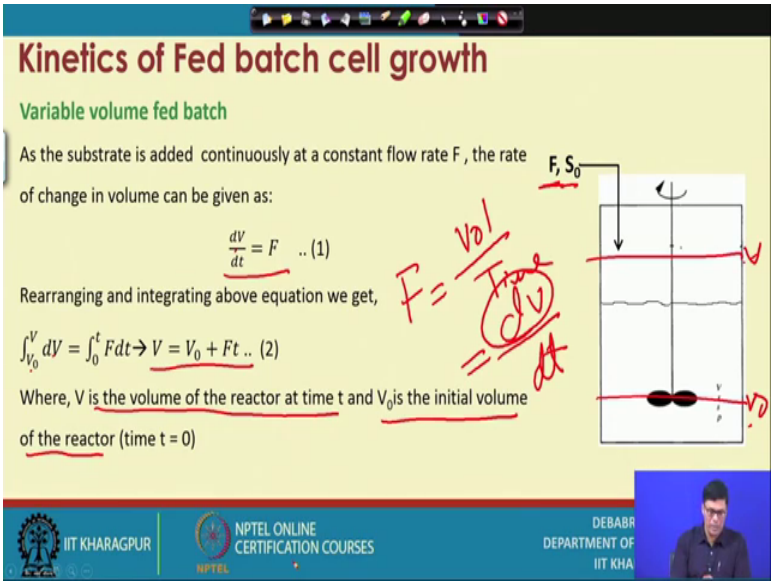

Now, question come how you analyze this system now, rate of flow that you know flow rate what is the unit of flow rate, what is the unit of flow rate if flow rate is equal to volume per unit time, am I right? So, this is exactly we have we shown here dV by dt this, we can write dV by dt dv is the change of volume, this is per unit time this is called flow rate. Now, we are adding this substrate at a flow rate F, and initial substrate concentration S 0.

So, what we can write change your volume what is this is initial volume might be you have V 0 and final volume you might be having V. So, V 0 to V that dV equal to 0 to the dt this is F into dt this, we can this we can write in this form and this is equal to v equal to V 0 plus Ft where, V is the volume of the reactor at time t and, V 0 is the initial volume of the reactor to be start with the V 0 volume and then, continuously added to make the volume up to Vt. So, this is how we can find out the volume of the fed batch reactor.

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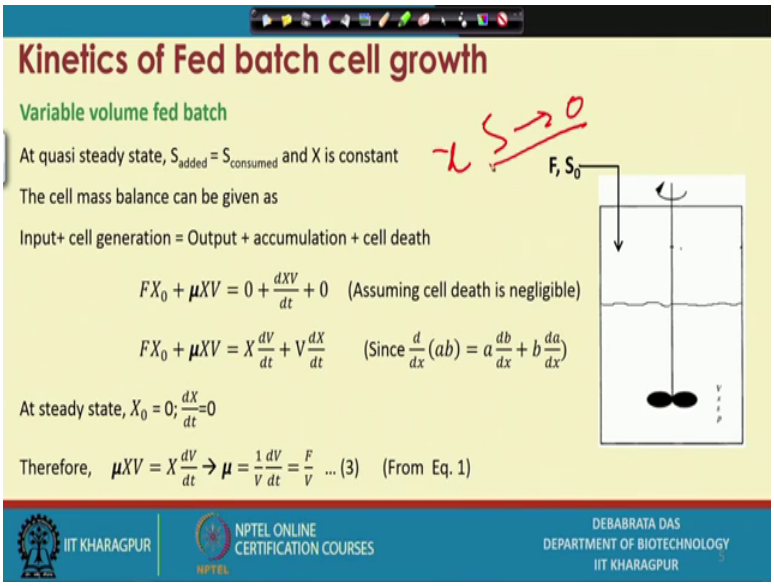

Now, let us say, let us discuss about the kinetics of the fed batch cell growth, how we can discuss the kinetics. Now, here, we can consider the pseudo steady state condition, what you I told you in my previous couple of lectures, that pseudo steady state means not exactly steady state, we assume it is approaching towards the steady state condition that is the pseudo steady tends to.

As for example, sometimes we say that x suppose we see, we always say that x tends to or S tends to 0, x tends to 0 does not mean say is equal to 0 is x tends to 0 it tends to 0. It is say something similar that you know pseudo steady state also like this, it is tends to it condition now, quasi steady state condition is a added surface added equal to surface consume. So, if you do that then and only then, you can you can keep on adding the substrate and because, you have to maintain the substrate concentration below the inhibition level suppose, the above this level we have substrate inhibition level.

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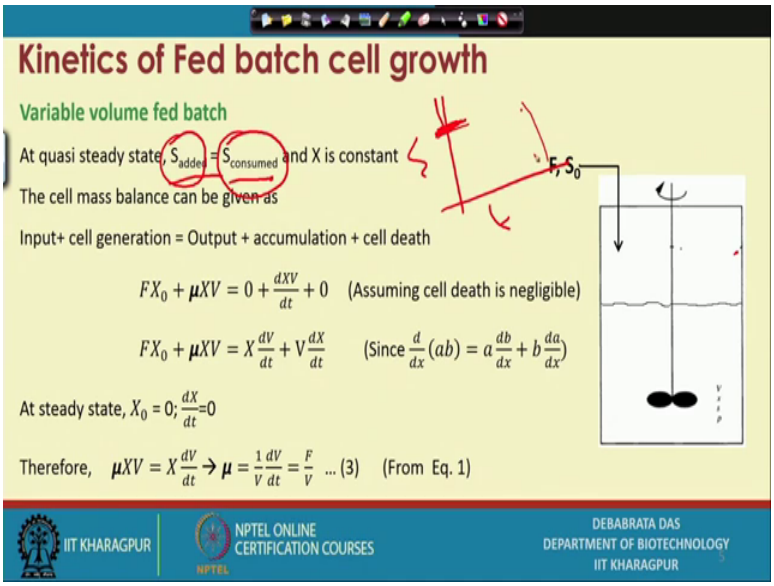

So, to maintain this inhibition level we shall have to add the substrate in a manner, that what about the substrate that is consumed that substrate you have to add. So, that these are and final volume reach then, you stop the feeding then, substrate concentration will keep on decreasing with respect to time. So, the when. So, what you can write the mass balance how we can write, this is the rate of input equal to plus rate of cell generation equal to rate of cell output plus rate of cell accumulation and cell death. Now, if you consider this is the steady state condition, this is if you consider that down steady state condition then because, you are adding you are not taking it out from the system. So, what is the rate of input F into x 0, this is here, let us assume this is the x 0 that this is what is the growth, that will take place mu into x at the dx by dt.

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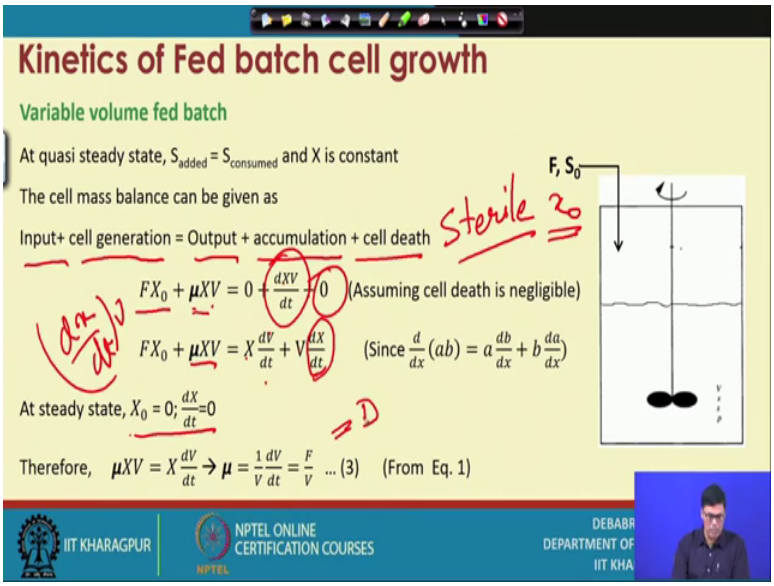

So, this will be dx by dt into V, am I right? This is the volume will be take place the output will be 0, there is no output in the fed batch reactor and rate of accumulation everything is the accumulative in the system cells are accumulation, can be expressed like this, and we can we can assume that, there is no cell death occur in the system.

Then, we can assume, this is the x is equal to 0, when we have sterile fed if I sterile fed then, we can assume there no cell present in the media then, we can assume that this is equal to 0, and then this equal to then we differentiate this x and a x and b then we can x dv by dt and to be dx by dt now, dx by dt if we assume, rate of cell formation that is the assumed to be 0, that is not the changing then constant then, we can write this is equal to x into dv by dt. Now, this is equal to mu 1 by V dv by dt equal to a by V. So, F by V. What is F by V is the dilution rate, that we have already seen this is nothing but equal to the dilution rate.

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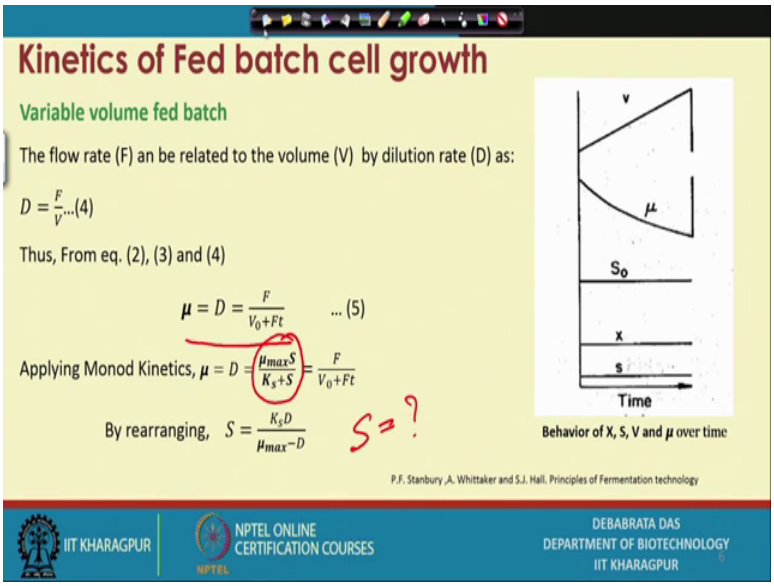

Now, again now here, we have what we have D equal to. So, this is equal to we can write that mu equal to D a by V0 by Ft, this sequence you write then from the Monod question. What is mu equal to mu max S, k S plus S. So, we can bring this and we can have we can by using this equation, we can easily find out what is the states the what is the substrate concentration at time t, that we can easily find it out here.

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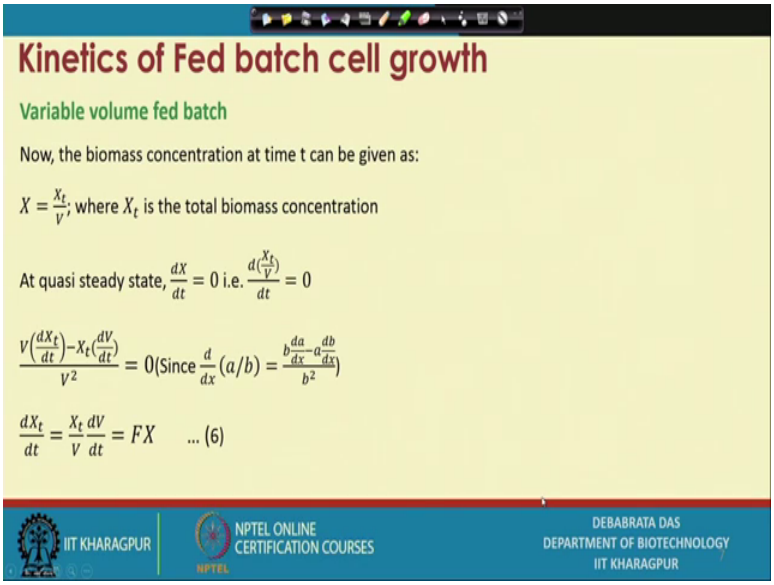

Then, now, the biomass concentration at time t can be given by x is the total cell mass, and x is the cell mass concentration then, xt is the total cell mass divided by volume is

the cell mass concentration. So, quasi steady state conditions the dx by dt we assumed to be 0 then, we can write dx t by V by dt equal to 0 then, we can differentiate this we will get this equation and this is the formula that we have and then, this equation we can write in this form dx t by dt equal to xt by v dv by dt equal to F into x, this we can analyze and we can write like this.

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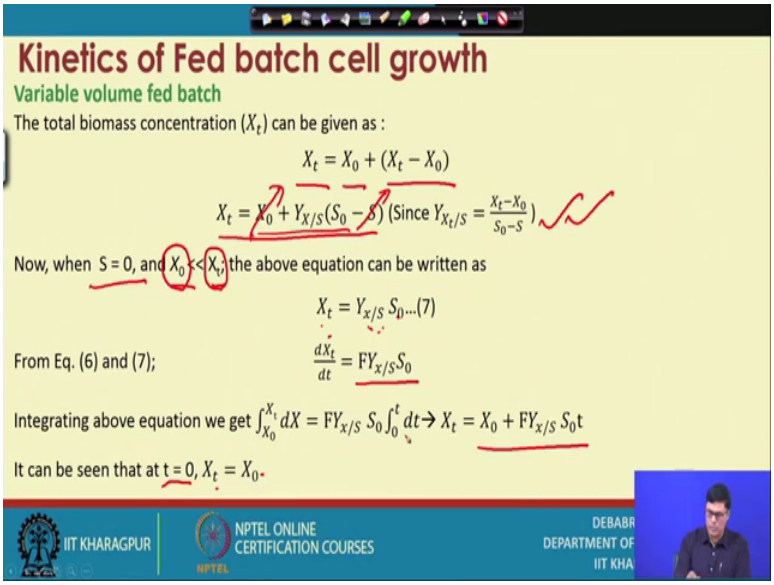

Finally, we find that x total is equal to x 0 plus a this we can write, xt equal to x 0, xt minus x 0. So, this we can also, we can write in the form of this also, this we have seen before also that this is a how it is related, with the substrate concentration this we have done before with the help or bill coefficient we can find it out.

Then, now, when S equal to 0 and x 0 is much less than xt. So, x t is the total cell mass concentration is very high as compares to initial cell mass concentration then, this equation when this equation we can write this we can neglect. So, we can write xt equal to this also you can neglect then, xt equal to Y x by x into x 0 then, this equation if you combine we can dx t by dt equal to F Y x t by S 0 .the integrating in this form we can have this equation x 0 plus if Y x by S, S 0 into t now, this is the boundary condition we can say at t equal to 0 xt equal to x 0. That that condition we can have.

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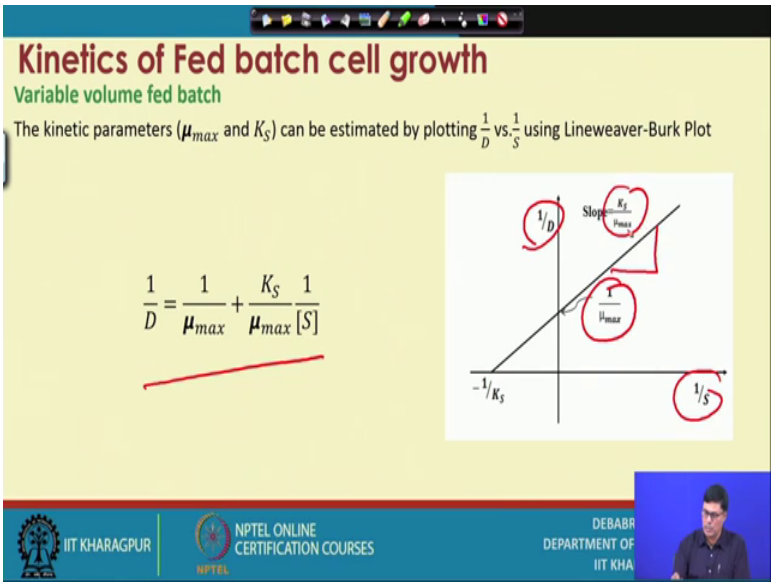

Now, how we can find out this kinetic constant by using the Lineweaver Burk plot, we can do that that we can we can put 1 by D versus 1 by S because, by this Lineweaver Burk plot by using Monod equation. We can find out the intercept we will give you the value of 1 by mu max, and slope will give you the k S by mu x. So, this mu I value you put it here, you will get the k S value of the organism.

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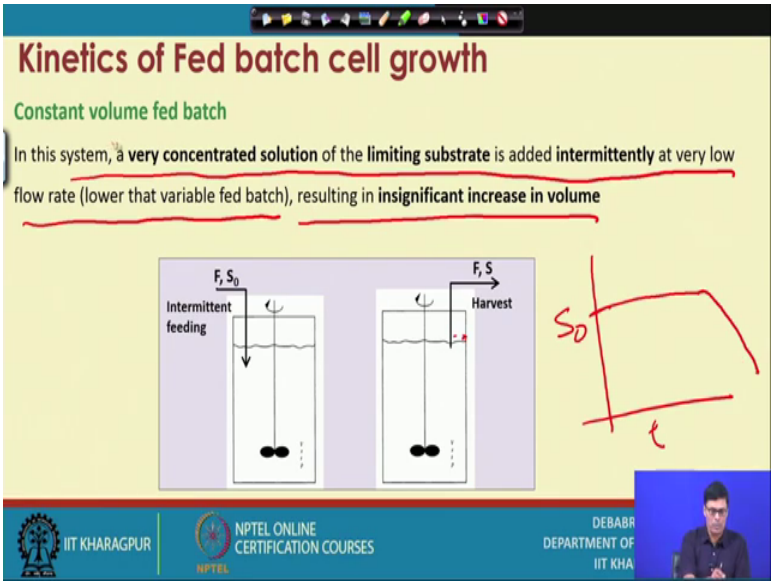

Now, let us discuss that, another interesting feeding strategy that, we have with the constant volume batch process. So, what do you mean, that we are here, in this in this

case what is to doing that a very concentrated solution of limiting substrate is added intermittently at the very low flow rate and what then, the variable fed batch process resulting in significant increase in volume.

So, what does it mean, means that in on that volume we are not increasing much, only we are increase, we are adding the concentrated, you know substrate solution, just to maintain as I told you that this S 0 value, we want to maintain constant, with respect to time. So, you keep it constant, and then when it reaches the final volume then, it keep on decreasing like this. So, so this is the constant volume fed this is the another strategy that we had in the fed batch process.

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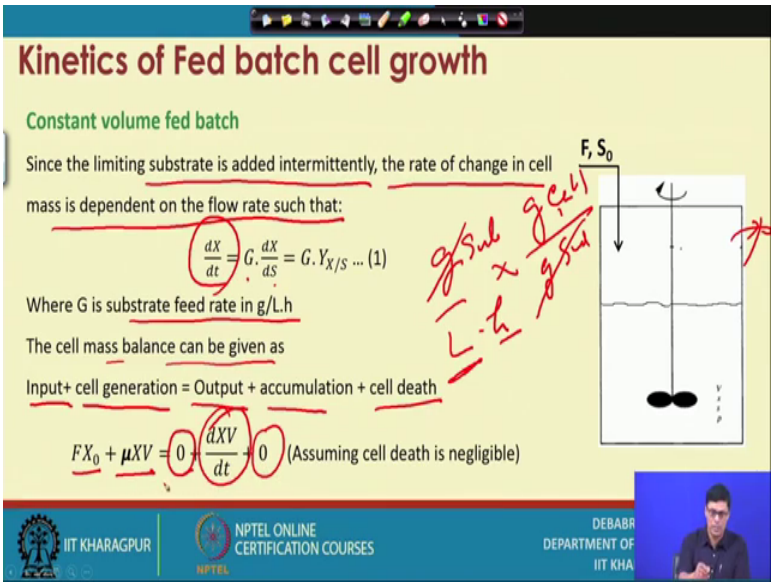

So, here, the analysis we can do like this since, the limiting substrate is added intermittently, and rate of change of the cell mass is depends on the flow rate such that, dx by dt equal to G into dx by dS, equal to this is G into Y xS what G, G is the substrate feed there is a gram per liter per hour that is the. So, you have gram per liter per hour.

Now, what is this yield, this is the gram of substrate am I right? So, what is this yield this is the gram of cell, per gram of substrate. So, this substrate will cancel. So, you will give it the gram of cell produce per liter per hour this is what we mentioned that dx by dt. Now, cell mass values can be written as that, we have already know shown this several times the rate of input equal to plus cell generation equal to, rate of output accumulation and cell death.

Now, here, we can write F into x 0 we have shown before then, this is the rate of cell mass growth the output is 0 no output here, this output is totally 0 and this is the accumulation of the cells, and we assume no cell death then, we can write this equation in this form.

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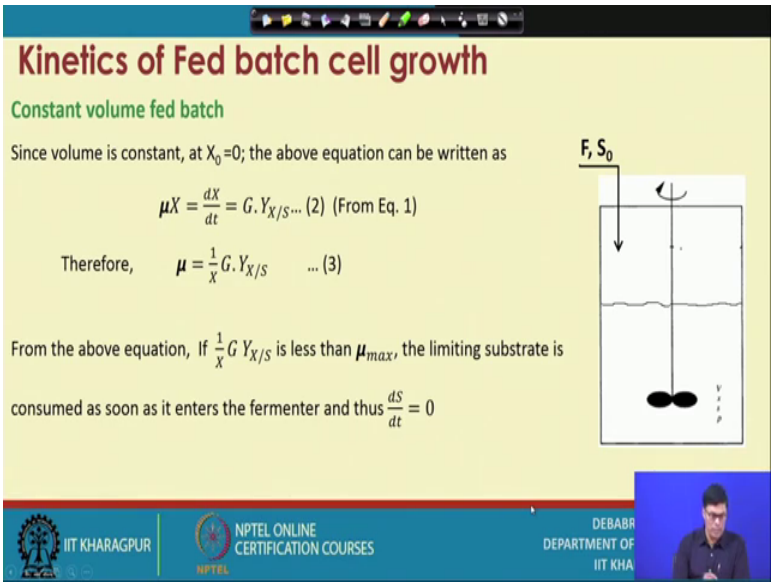

Now, if you look at this equation then, we can bring it bring it back in this form that the volume, if we assume the volume is constant now, x 0 tends to 0 then, and then the above equation we may be, written as mu x equal to dx by dt equal to, G into Y by x, the mu will be what 1 by x G Y x by S.

Now, 1 by x G 1 by is less than mu max, the limiting substrate is consumed as soon as, it entered into the fermenter thus, the ds by dt equal to 0 though, this is the assumption, we can rate that when this equal to less than, the mu max value the limiting substrate I explained, that limiting substrate which control the growth of the cell as soon as, is the enter in the fermenter and just dS by dt that will be equal to 0.

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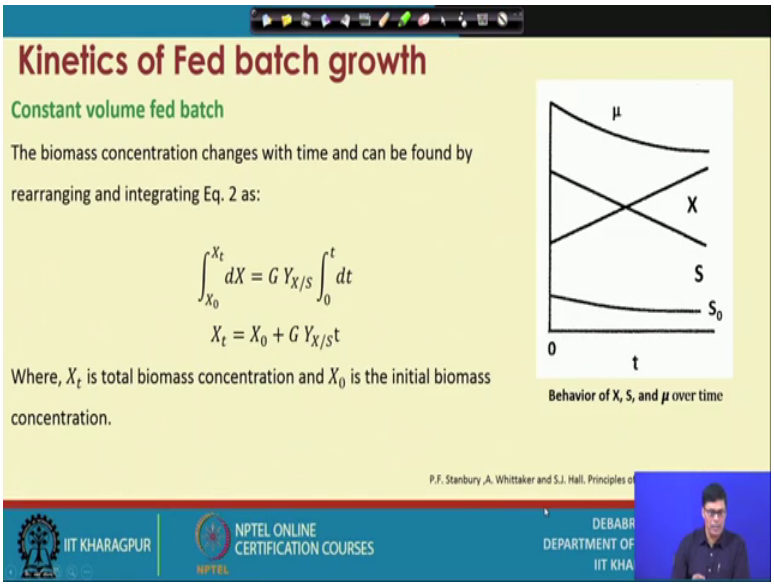

Now, finally, we have this equation, if you look at previous equation is like this then, this equation we have then, we can write this equation the dx equal to, G into Yx by S and to and dt, and this is integrate from 0 to t then, in this particular system though we can have xt equal to the total cell mass concentration with x 0 plus G into Yx by S into t. The xt is the total cell mass biomass concentration, x 0 is the initial biomass concentration. So, this is how we can analyze this system.

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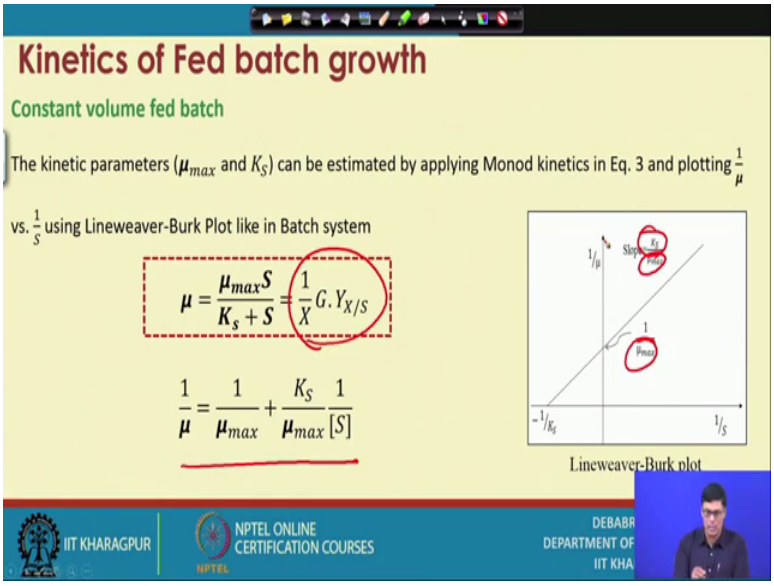

Now, that kinetics that equation that if you when we take the Monod equation here, mu equal to mu max is this can be equal to, 1 by x G into Y x, and we can write again in this form, and from this we can find out the value of this kinetic constant mu max and k S, we can we can calculate.

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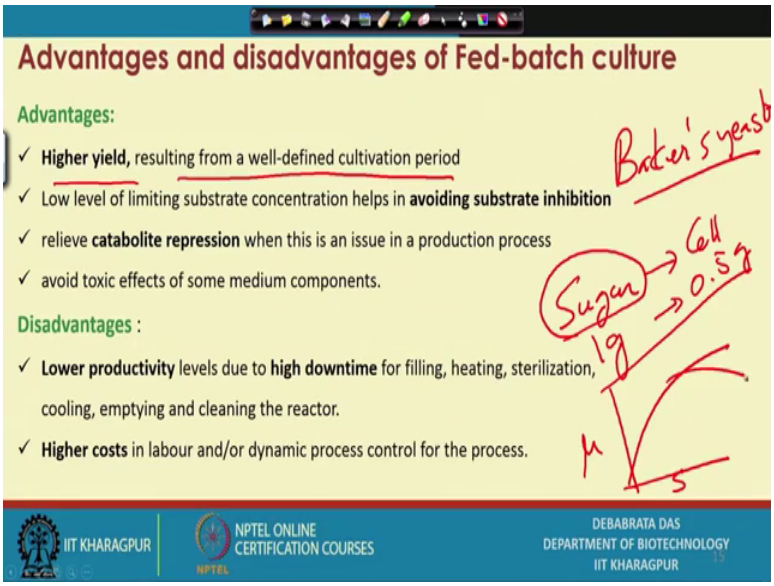

Now, advantage question comes, what are the advantage and disadvantage of the of the head batch process? The higher yield resulting from the well-defined cultivation period. So, because I can give the example, the bakers yeast fermentation process. Baker's yeast fermentation process now, in the Baker's yeast fermentation process, what is the stoichiometry we have? We have suppose, we use the sugar as the raw material and sugar converted to cell, am I right?

Stoichiometry is that one gram of sugar, usually converted point 5 gram of cells. So, we want to increase the amount of cell production. So, what we shall have to do, we shall have to use the amount of sugar input to the system. Now, we have seen before that as you substrate increases in a batch process, if you increase the substrate then, we find that kind of substrate inhibition, mu way plot we this is no inhibition and then, we have some kind of substrate inhibition that tale place.

Now, question comes; that means, if you as soon as you go for the highest substrate utilization then, that you know the rate the activity of the organism that will be reduced to a great extent. Now, to avoid this situation this fed batch process appears to be the

better solution. The reason is that since, when since you are adding in a stripping wise manner. So, amount of substrate that is the utilized in the system will be more as compared to batch process.

Batch process, the same batch process same volume, if you use and you and compared with the fed batch process, you can use mode amount of substrate. Since, you are using mode amount of substrate. So, you will get the more amount of cell mass in the system. So, one particular application we have that is in case of beakers is, the another example is the penicillin fermentation process.

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In the penicillin fermentation process what is happening, that one mole of we know the precursor that is required in the penicillin fermentation process, the phenyl acetic acid and phenoxy acetic acid, am I right? Now, though you know this is one mole of phenyl acetic acid produce one mole of that penicillin G, and if you know one mole of phenoxy acetic acid produce one mole of penicillin B.

So, naturally if you now, a since, this is the acid if we add all this acid at a time, it will give some kind of inhibition effect on the growth of the cell, that is a major drawback. So, that a particular problem, that you know that there that, if you want to produce more penicillin. So, naturally fed batch process might be the one of the major approach.

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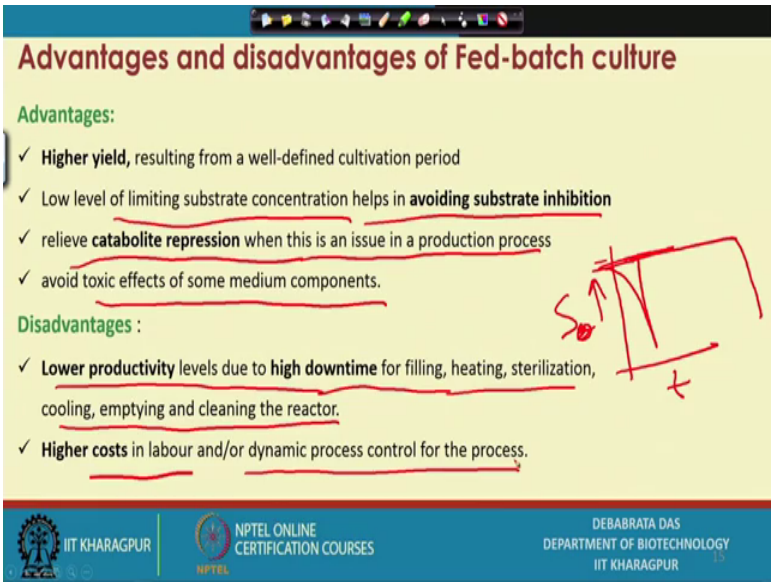

The low level of limiting substrate concentration, helps avoiding the substrate inhibition. That is the, this is the major facts that we have what I try to point out, this is substrate concentration and this is time show, we maintain a particular level of substrate is below the inhibition level then, you keep on feeding and show that here your rate of reaction does not happen.

The relieve the catabolic repression, when this is an issue in the production process the catabolic repression that problem also can be overcome, avoid the toxic effect of some media components. So, that also can be avoid to some extent because, you are not adding, you are adding only the substrate that in the stoichiometry on the basis of stoichiometry you are increase, you are not adding, all the components present in the reaction mixture or in the in the media.

So, necessarily the possibility of toxic accumulation that will be reduce, that this advantage of this process and lower productivity level, due to low high downtime for feeling, heating and sterilization, cooling, emptying, and cleaning the reactor. So, it will required lot of time for you know that the downtime will be more, and higher cost in labor and dynamic process control of the process is required. So, this process is not as simple as your batch process, in the batch process we have seen, they we take the material at a time allow it to react, after this reaction is over you take it out.

The this is something in combination of the batch and continuous process. But here, we started with the batch mode then, continuously we feed the material in such a way that, your substrate concentration all is the remain below the inhibition levels. So, that your mole product formation take place and you get the products.

So, in conclusion what I want to say tell you that, that as compared to that you know batch or chemostat or plug flow reactor, the fed batch has some added advantage, added advantage in the same that. That we can use more amount of substrate in this particular fed batch process, and the substrate inhibition problem that can be avoided to some extent.

There are 2 type of strategy that we have, variable volume or fixed volume through which, the fed batch operation can be done, and we showed you the how the kinetics of this process can be analyzed and finally, we discuss what are the what are the merits and demerits of the fed batch process.

Thank you.