Aspects of Biochemical Engineering
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Lecture – 37
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – VII

Welcome back, to my course aspects of biochemical engineering and last couple of lectures we try to cover the theoretical part, then analytical part of that microbial system hide the kinetics of substrate utilization product formation and biomass formation by using different microorganism can be explained and what can be analyzed.

So that, we started with that, you know how we can monitor the cell mass concentration, then we discussed the what are the different models, we have we discussed the structure model, unstructured model, segregated model, then we discussed about the inoculation of the microorganism using the life cycle, then we analyzed the batch process we analyzed the continuous process as per continuous processes is concerned, there are 2 type of process one is chemostat or cstr and another is plug flow reactor and another process we explained, that is the fed batch process which is mostly used for the where, the substrate access the inhibitor.

Now, after that we discuss different type of models, we discuss the chemostat process in details the major drawbacks of the chemostat process is the cell mass wasting from the reactor, if it is more has compared to the cell mass, that is growing in the reactor; obviously, that a time will come and there will be you know self-pressure in the reactor though the situation what we call d washout that will arise.

So, we try to discuss what are the avenue through, which we can safeguard this particular process one approach is, it is cell mast recycling another is the immobilized immobilization of the whole cell.

So, we discussed about the processing details, that cstr with cell mass recycling and also whole cell immobilization system, how it is used in different how those process used in the industry or different biochemical processes.

Now, today our purpose is little bit different, we want to discuss it. I say with the couple of lectures we will be concentrated on the different numerical problems and I personally feel that, more we solved the mu and numerical problems your idea on this particular biochemical process will be clear.

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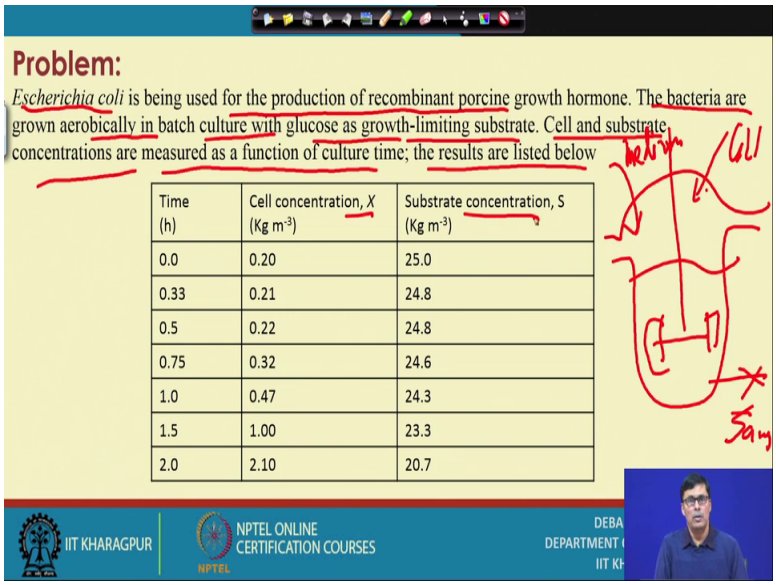

So, with this objective the first problem, that we have taken into consideration, that is the batch process, because you know that when cell grow in a particular system, that question comes how you can monitor the kinetic constant? As for example, maximum specific growth rate of the cell and the saturation constant.

Now, this problem is little bit deal with this, you see that a Escherichia coli is being used for the production of recombinant this porcelain cell growth hormone the bacteria are grown aerobically in batch culture with glucose as the growth limiting substrate and cell and substrate concentration are measured the function of culture time and results are listed below. So, you know we understand what is called batch process, batch process I told you we take the we will take the material at a time, we take the media, we take the medium and we put the cell here.

So, you know than, we put all the things together and then, time to time we draw the sample here, and we monitor with respect to time what is the cell mass concentration what is the substrate concentration.

So, this is exactly this problem deals with like this, and these are the different their experimental results that has been given then, what is the finally, for their laxing for that, what is the value of mu max and KS what do you call maximum specific growth rate and the saturation constant KS and what is the observed biomass yield of the substrate, that is the these are the 2 this is the parameters we select to monitor.

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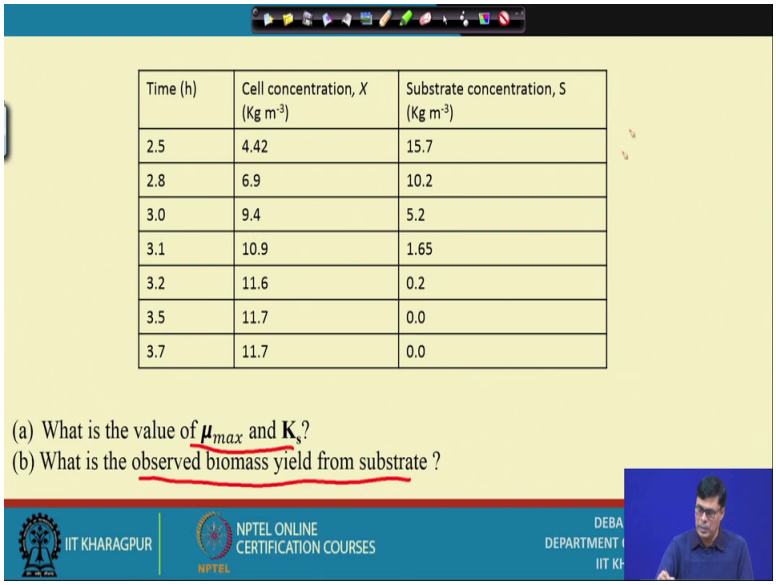

So, we know that, to solve this problem we shall have to take the Monod equation. Now, we know the Monod equation, what is the Monod equation? Monod equation is mu max, mu equal to mu max S KS plus S, am I right?

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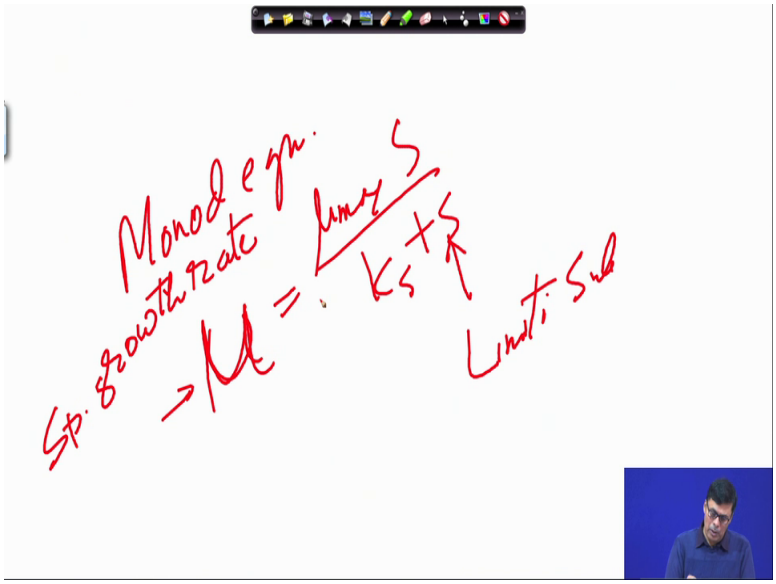

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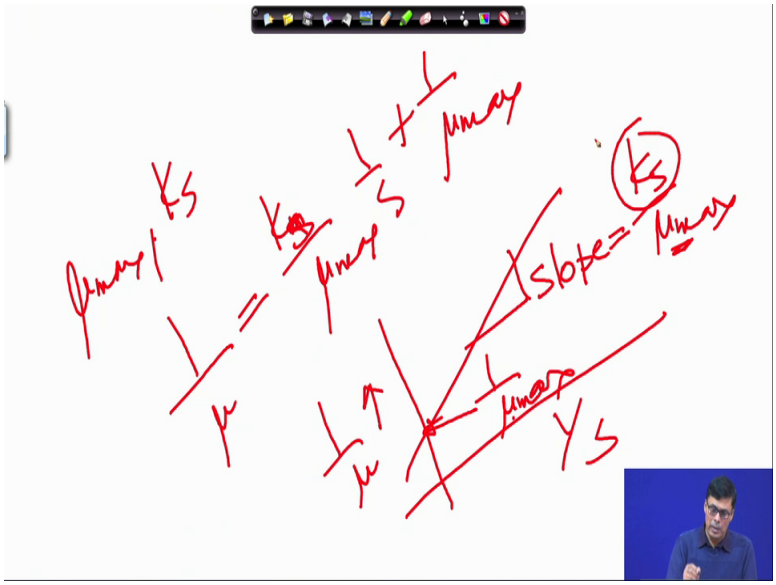

Now, mu what is mu? Mu is the specific growth rate, this is called specific growth rate and what is this S is called the limiting substrate. So, the ones what we shall have to do to solve this particular to finding out to find out the values of mu max and KS, what we have what we shall have to do we shall have to put the line light the Lineweaver Burk plot using the Monod equation and this will be, what KS this is KS by mu max 1 by S plus 1 by mu max, am I right?

So, if you plot now, 1 by mu versus 1 by S will get a straight line the slope will give you the value of this is KS by mu max and this intercept will give you the value of 1 by mu max, am I right? So, this is how we can find out. So, this mu max value you can put it here, we can find out the value of KS.

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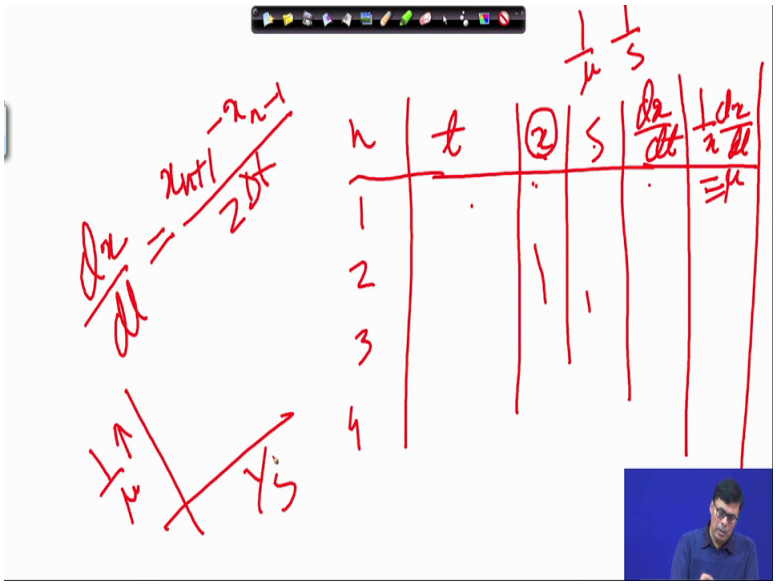

Now, question come how you can find out the value of mu. So, in this problem what you have we have t we have x and we have is value. So, different value in this problem is given now, what we shall have to find out we shall have to find out what is dx by dt now, I told you in the dx by dt how we can find out, this is equal to x n plus 1 minus x n minus 1 divided by 2 del t.

Now, here n is the sampling number, this is sampling number 1 2 3 4 5 6 like this. So, simple. So, by using this equation we can find out dx by dt, once we know dx by dt we know the at this point what is the value of x and we at this point we know the what is the value of dx by dt. So, we can find out 1 by x, value of 1 by x dx by dt, this is nothing but mu, am I right? And once you know the mu value then we can find out the value of 1 by mu and simultaneously, we can find out 1 by S value is given here.

So, then we can plot 1 by mu versus 1 by S and we can get the kinetic constant KS and this we can will be find out.

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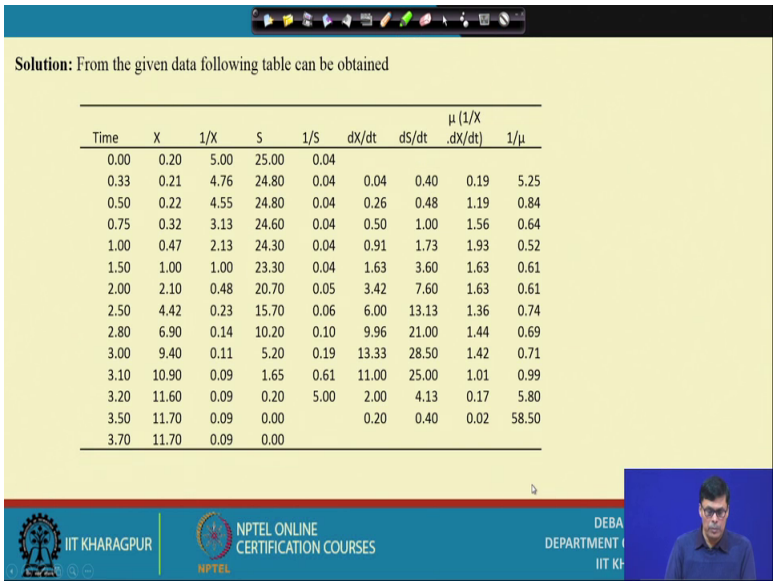

Now, this is this is the table that you can see that, and different values are given this is the x value 1 by x value dx by dt this is how we can find out, and then we find out the mu value, which is nothing but 1 by x dx by dt, then 1 by mu value we can find out.

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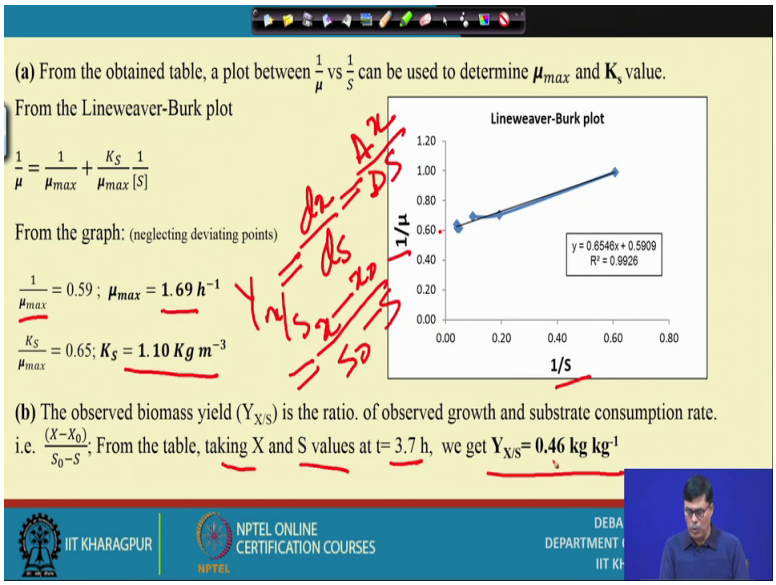

Then we can plot this is 1 by mu versus 1 by S plot, then we will get a straight line and from the intercept we can get the value of 1 by mu max, that is coming 1.69 our inverse and the slope is 0.65 you put the value of mu max here, the mu will get the KS value 1.1 Kg per meter cube.

So, we can find out the kinematic constant now, question comes that it makes is the what is the observed biomass yield the ratio. So, what do you can write that, YX by S is equal to dx by ds, am I right? And this we can write this is equal to del x by del S though, this is equal to x minus x 0 by S 0 minus S. So, we can from this ratio we can find out that, from the table x we can take a taking the value at time 3.7 hours we get the YX by S value 0.46 Kg per kg.

So, we can easily find out the values of the different kinetic constant of any microbial flow system, I will hope it everybody can do it very easily.

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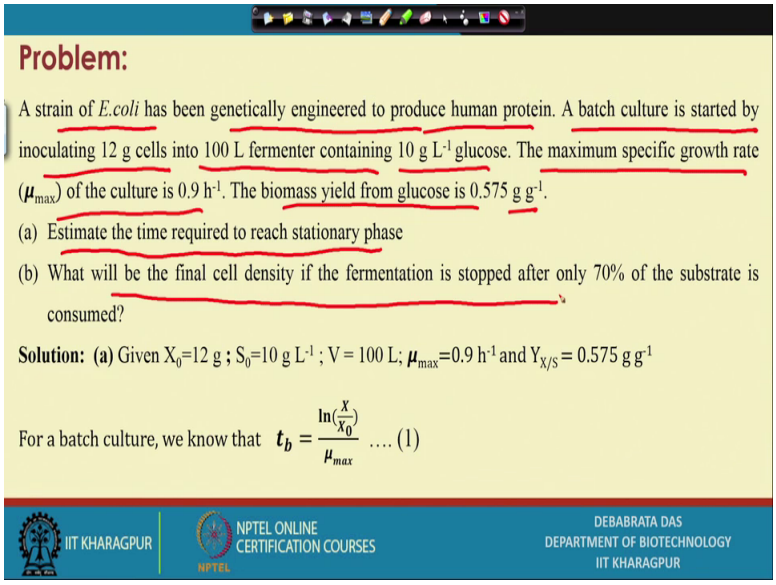

The next problem, that is very interesting, that is also with respect to the batch process and this problem is interesting the reason is that, because the, we are when you done a particular batch process we had interested to know that, you know after certain time what is the cell mass concentration? What is the substrate conversion? So, the how you can monitor? How we can determine this though this program will deals with that.

Let us see, how we can solve it now here, a strain of E.coli has been genetically engineered to produce human proteins you know that, one human protein is the insulin, that is by this is a the recombinant protein that, we produced through the microbial fermentation process and this a batch culture is started by inoculating 12 gram of cell into 100 liter fermenter containing 10 grams per liter of glucose the maximum specific

growth rate of the culture is 0.9 our inverse, biomass yield from glucose is 0.575 gram per gram.

So, first problem, that we have estimate the time required to reach the stationary phase and what will be the final cell density, if the fermentation is stopped after 70 percent substrate with consumed though, this is very typical this is a common problem, that we have with the batch process that, we are interested to know, that the how much time is required for the attending the stationary phase.

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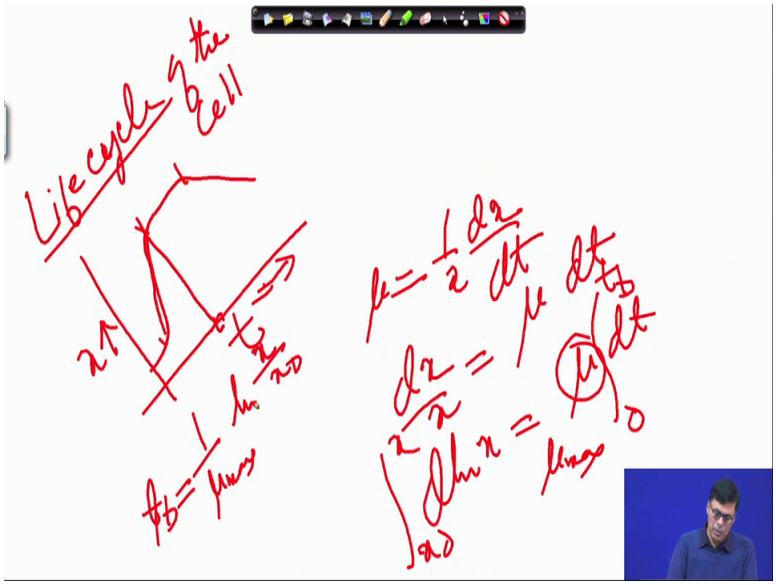

Can I can explain like this, if you know the life cycle, life cycle of the cell. So, what is the life cycle of the cell this is the x with the cell mass bible cells and this is time. So, we will be having this. So, this is lack phase, this is log phase, this registration phase and this is depth phase.

So, the question is that, that we had in this program what is the time required to reach the stationary phase. So, we shall have to find out this value t1 now, question come how we can do that? How you can find out that? Because, you know that, that we shall have to find out now, you we know that, what do you know that, mu equal to 1 by x dx by dt, am I right?

So, dx by x, I can write mu into dt, this is dt and this is dln x mu into dt, am I right? Now, this is equal to x 0 by x and this is 0 to t now one thing here, we can assume they are

saying that, then after the end of block phase that, what is the cell mass concentration we can assume, this mu equal to mu max the maximum growth rate of the cell occur in the lock phase.

So, if we assume that, then the (Refer Time: 13:18) situation is very simple we can write this is the t batch process, you know t batch will be, what t batch will be equal to 1 by mu max and ln x by x 0, am I right? So, this is exactly, what we have done in this problem. So, this is the equation, that we had we have written here, this is ln t tb equal to ln X by X 0 by mu.

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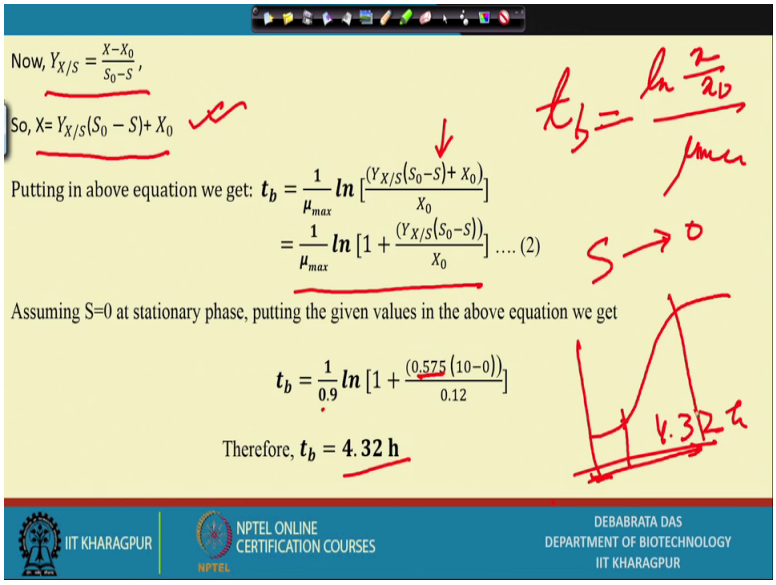

Now, once you have this we know that, this is the yield coefficient is Y X by S is equal to X minus X 0 by S 0 minus S the, so X will be equal to what this YX by S S 0 minus S plus X0. So, we have what are the tbs equation, tb equal to ln X by X0 divided by mu max, am I right?

So, here 1 by mu max I can take it out now, X will be equal to this. So, I can put this value here, and this is the X0. So, we can simplify in this form and then, we know we assume that that the stationary phase, there is what is the stationary phase? How you define stationary phase? We define stationary phase1 is the kind of starvation phase where, most of the substrate will be exhausted, am I right?

So, we can assume that, S tends to 0 not exactly, 0 S tends to 0 now, if it is tends to 0 I can assume I can solve this problem like this is 1 by 0.9 if the 0.9 is the mu max value then, YX by S is the 0.575 and 10 grams per liter is the substrate and this is 0 and X 0, if the initial cell concentration is 0.12. So, total time required for the batch process will be coming and 4.32 hours; that means, after if you look at this is the how long is the time required to use the stationary phase, that you know, that we assume is starting it is start in the this lock face initially and find them this is 4.32 hours, that you know that we can we can use this stationary phase.

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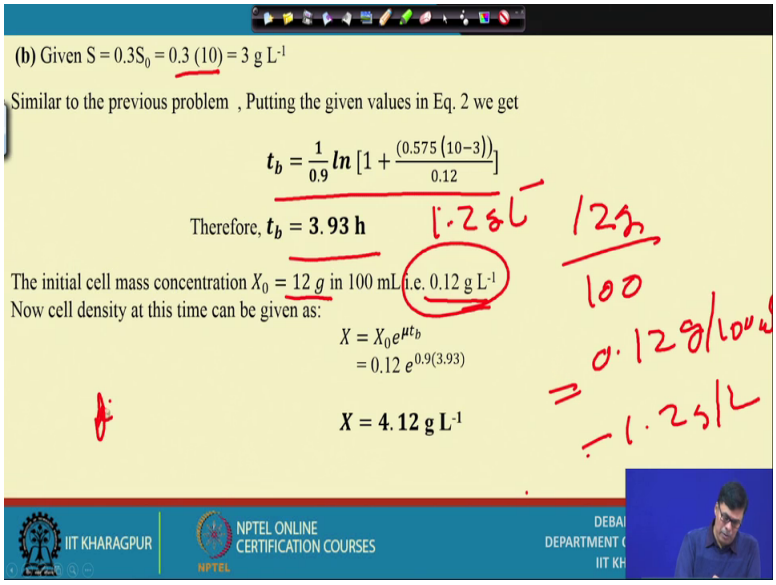

Now, next is that, you know what is the cell mass concentration? Now, if you look at this problem that, what will be the final cell density? What do you mean by density? Density mass per unit volume, if the fermentation stopped after 70 percent substrate it consumed. So, this problem; that means, we shall have to find out when 70 percent substrate consumed the how much was the initial substrate 10 grams per liter, the how much substrate will be remaining with us this is 30 percent, am I right?

So, you multiplied by 0.3; that means, 3 grams per liter now, until you have, we have the previous equation I can put the value of S and instead value S in this equation and find out, what is the time required to consume the 70 percent substrate it is coming 3.93 hours.

Now, initial substrate, that cell mass how much there 12 gram per 100 milliliter show this if we divide by that, you will get the 12 that is, this will be how much? The you see 12 gram per 100 grams, am I right? So, this will be above 0.12 gram per 100 milliliter. So, per liter it will be 1.2 gram per liter. So, it has little bit error is there, you please correct it this will be 1.2 gram per liter, am I right?

So, then we know that, dx we have already seen that, ln X 0 by X we can write this is X by X by X 0, this will be equal to what we have seen this is equal to mu max into tb, am I right? Time of the batch process. So, I can write X equal to what X0 into e to the power mu max into tb, that you know how much time required to 70 percent, this is exactly done only this value will be changed and this value will be little bit altered, that will that on later on will correct this.

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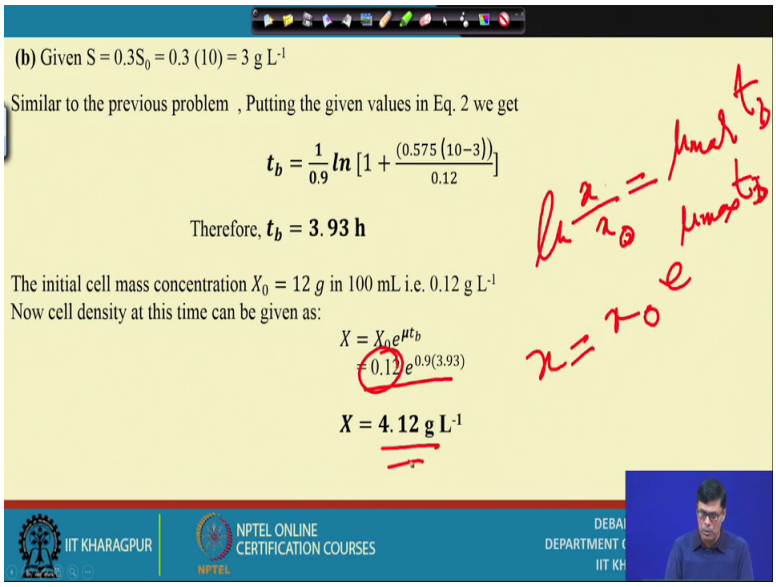
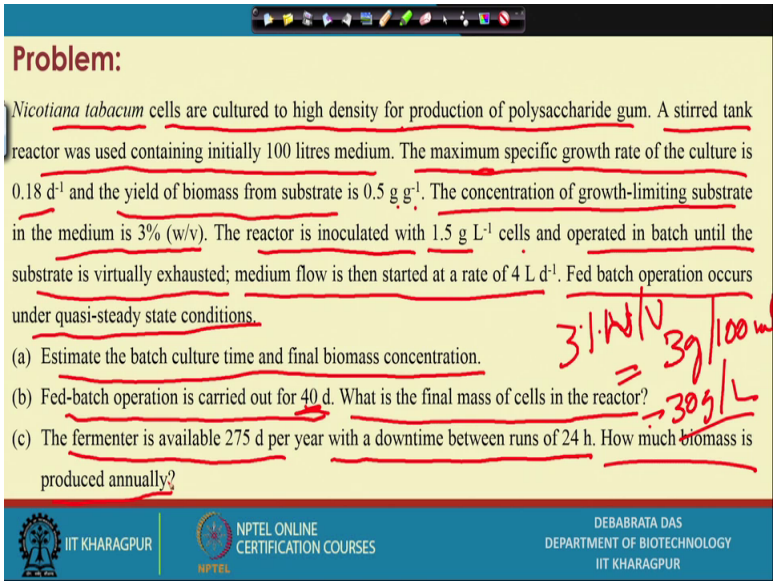


Now, next problem deals with the fed batch reacted by told you the fed batch reacted is lastly use for substrate inhibition and let us see, how we can analyze this process is very interesting problem. Now, if you look at that, nicotiana this is tabacum these cells are cultured in this in a high-density production of polysaccharide gum a stirred tank reacted was used containing initially 100 liter of media.

The maximum specific growth rate of the culture is 0.19 they inverse and yield of the biomass is 0.5 gram per gram of substrate. Now, concentration of the growth limiting

substrate is 3 grams, 3 percent weight by volume now, 3 percent weight by volume means, what? Did I can write 3 gram per 100 milliliter, am I right?



So, this will be 30 gram per liter. So, you know this is the how you can right, there the reacted is the inoculated with 1.5 grams of per liter of cells and operating batch until the substrate is virtually exhausted and media flow, then started at a rate 4 liters per hour fed batch operation occurs until quasi steady state condition, under the quasi steady state conditions.

Estimate the batch culture time and final biomass concentration, second is the fed batch operation is carried out for 40 days and what is the final mass of the cells of the reactor, then fermentery is the available for 275 days per year we say, down in downtime between runs is about each run between the runs is 24 hours; that means, after one run is take about 40 days, am I right?

So, after 40 days, that you have 24 hour is the downtime and how much biomass is produced annually. So, this we have to calculate this with the I consider this very interesting problem.

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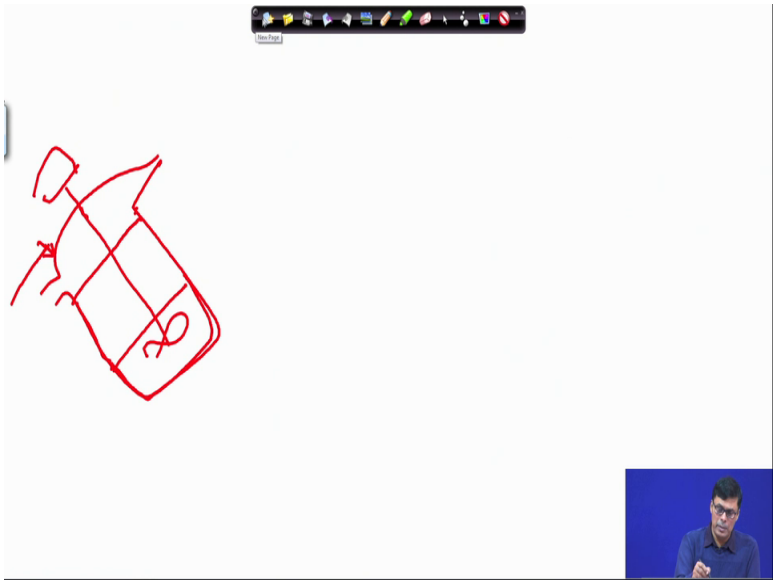

Let me explain this problem, actually what they are telling. So, we have this is you know that, in a fed batch reactor you take the initial culture you know this is the batch mode.

So, you take the media take the cell here, and let this organism grow in the batch mode and you operate the system until unless, that whole substrate with exhausted then, you continuously repeat the substrate and slowly, slowly this media is like this and you keep the substrate concentration you add in a mini managed show that, this substrate concentration remain much below the inhibition level, this is what we the purpose of the of the this fed batch operation.

So, we still have to find out how and they in how much cell mass is produce let us see, how we can solve it.

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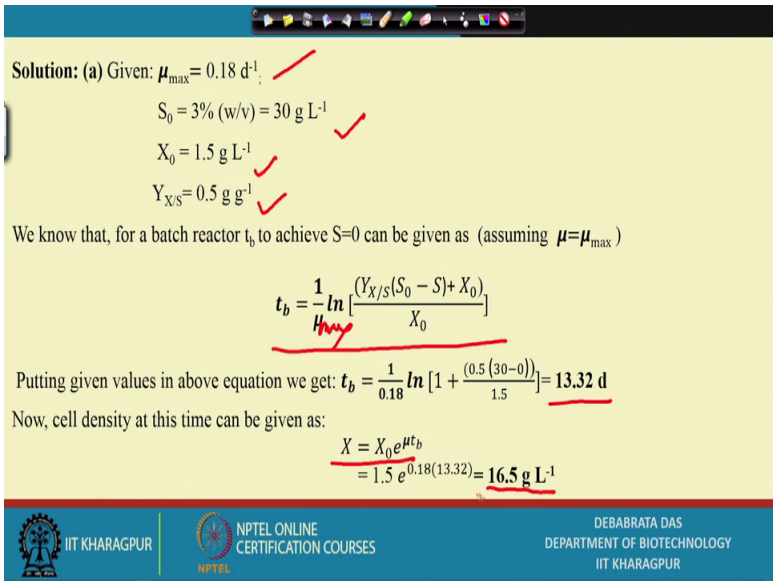

Now here, I told you whenever we try to solve any kind of problem first we still write down, what are the parameters are given in this problem. Now here, what is the parameters is given, minimize value is given, S0 value is given, then X 0 value is given and YX by S value is given.

Now, from the bbs problem we already found that, tb X equal to 1 by mu, that is to consider reconsider the mu max equal to YX by S S0 minus S plus the X 0 by X 0 you can put these values here, that we consider all subscript is exhausted. So, S we can consider as 0 then we find out that, how much exact time is required for this for this batch process we can find out that, will come around 13.3 to our days.

Now here, question comes what is the final cell concentration, that we have only to derive this equation in the previous problem and we can find out that, how this is 16.5 gram per liter will be the finals cell mass concentration.

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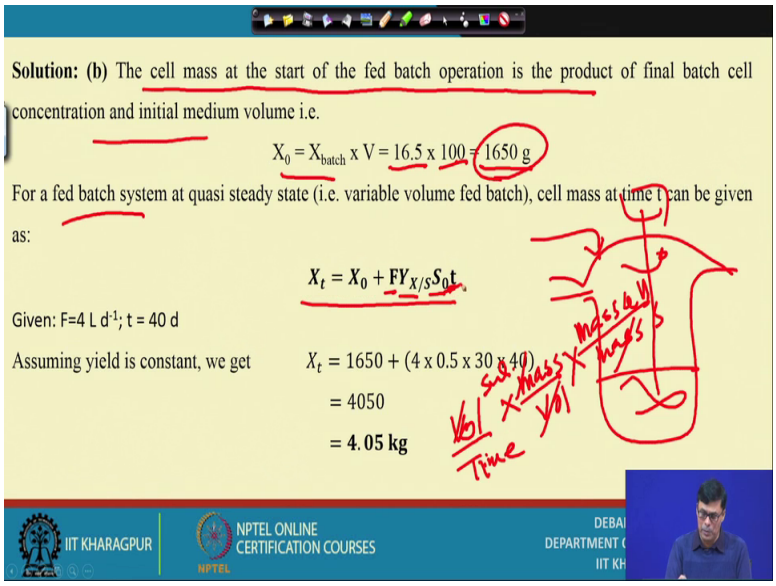

Now, next is that next what I told you hope you can remember that, you know that this initially we operated in a batch mode, this is a batch mode now, that this reacted that, we are now continuously feeding in the reactor. So, this is the strategy that we have.

The cell mass at the start of the fed batch operation is the product with the final batch of the cell concentration X0 is the X batch into volume is I put this. So, we know this is the concentration, concentration is the mass per unit volume and this is the liter. So, this is gram per liter and this is the 100 liter. So, if you multiplied that, we can find out what is the initial amount of cell present in the batch a final after the ending of the batch process.

Now, we in the fed batch process, we have already developed this equation what is the Xt? Xt is the final cell mass concentration Xt equal to X 0, F is the flow rate, YX by S is the yield coefficient yield coefficient, S 0 is the initial substrate concentration and t is the time.

So, I can write that, what is the volume volumetric flow rate volume per unit time, am I right? Volume per unit time and what is the value of S 0, S 0 is the mass per unit volume. So, this volume will cancel this mass per unit time. Now, again this is mass of water what mass of substrate you know this substrate and then YX by S is the Y you X by S is equal to what the mass of cell by mass of substrate mass of substrate. So, this substrate will cancel the mass of cell now, per unit time so if you multiplied by total time, then will get the what is the final substrate concentration this cell mass concentration. This is

the how this equation has come now, simple we put this value we can find out the at the end up the permutation we are getting 4.05 kg up.

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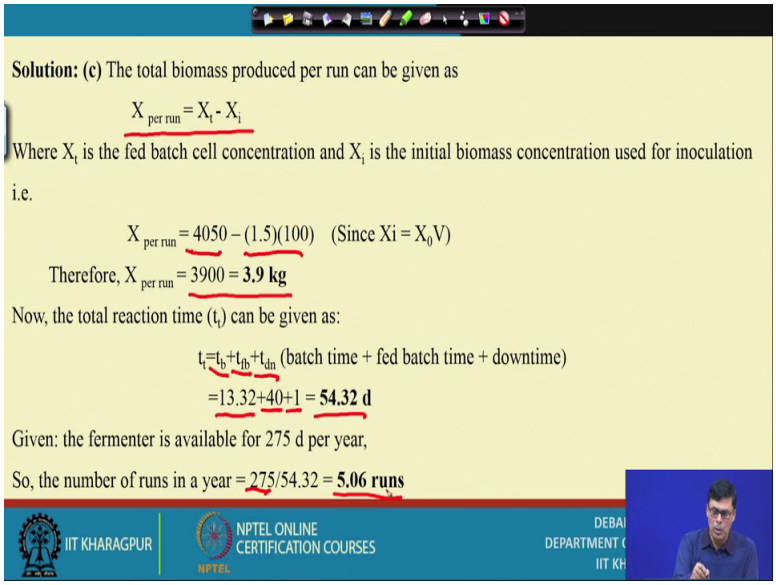

Now, let us come to the second part third part of the problem what is the third part of the problem, that the fermenter is available now, if you know the fed batch operation is carried out for 40 days, what is the final cell mass? So, that we have find out that, t we considered 40 days we find out and third parties that, fermenter is the available 275 days per year with a downtime between the runs is 24 hours, how much biomass is produced annually?

Let us see, how we can solve this we have what you can the per run is that per run, how much is the cell mass we can produce this is the total cell mass, this is the initial cell mass. So, what is the with the part run how much it this is the final, we previously find out how much is the cell mass concentration, we can find out it is 4.05 kg, that is mean 4050 gram and this is the initially cell mass that was present. So, if you if you multiply that, you will get that, how much cell mass you produce per run.

Now, how many runs you can conduct this is the best time, that this is a how much 13.32 and then, fed batch time how much is required 40 days and down time is required one with the total time is required like this. So, how much run because, we shall have to operate 275 days. So, divided by 54.32; that means, 5.06 run you have to conduct, am I right?

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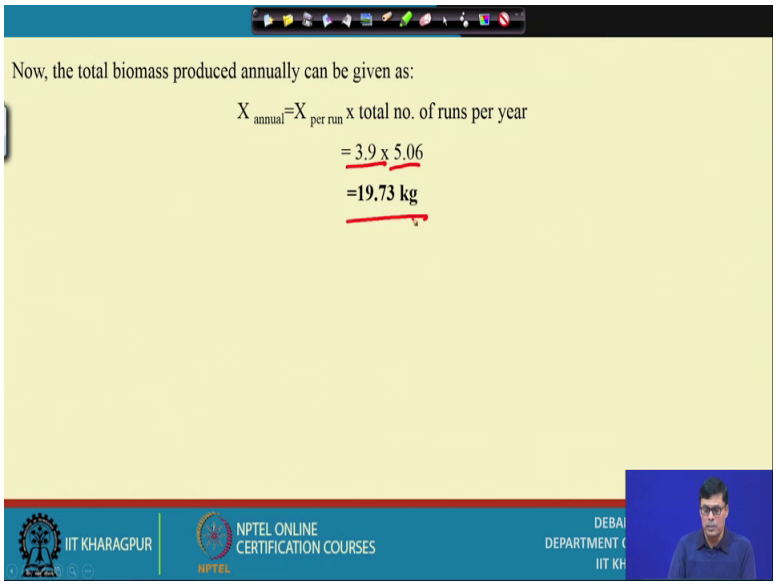

If one run we can produce 3.9-gram 9 kg uprooted it cell mass the 5.06 run, how much is cell mass is produced 19.73 kg. So, we can easily find out that total amount of cell mass is produce.

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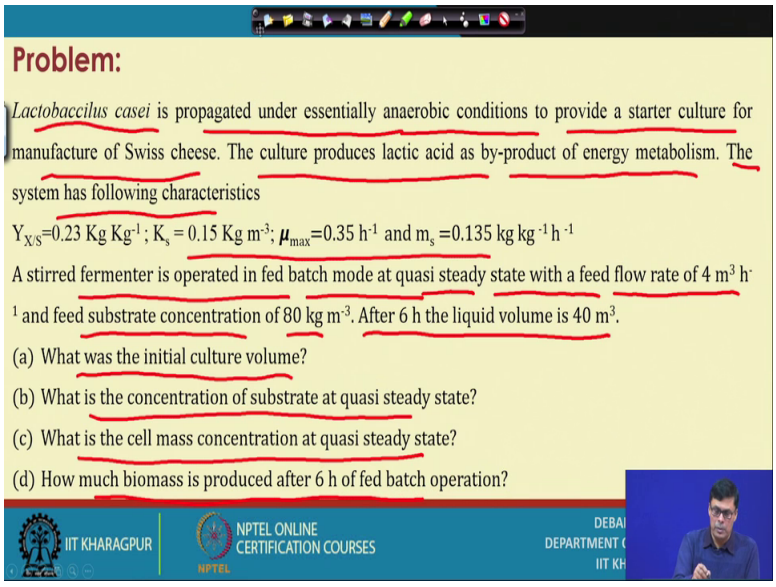

Now, last problem that we have lactobacillus casei, this is also very interesting by similar to fed batch process lactobacillus casei is propagated under the essentially anaerobic condition to provide the starter culture for manufacturing the Swiss cheese. Now, the culture produced lactic acid by product for energy metabolism. The system has the

following characteristics this has the different values are given, the stirred time reacted is operated in fetch be fed batch mode at quasi steady state with a feed flow is 4 cubic meter per hour and feed substrate concentration is the 80 grams per cubic meter even kg per cubic meter after 6 hours the liquid volume is 40 cubic meter.

What is the initial volume of the culture, that you have to find out what is the concentration of the substrate at the quasi steady state? What is the cell mass concentration at the quasi steady state? How much biomass is produced after 6 hour fed batch operation. So, I am quickly go through this because, so you see that we have already we have seen that, V equal to V 0 plus safety now, V 0 we know the initial volume we shall have to find out the V 0 V minus Ft. So, V is given this is 40 liter or 40 cubic main meter, then F is the 4-meter cube per hour and this is the 6 hours time. So, we can easily find out the initial volume, initial volume is very easy you can determine that.

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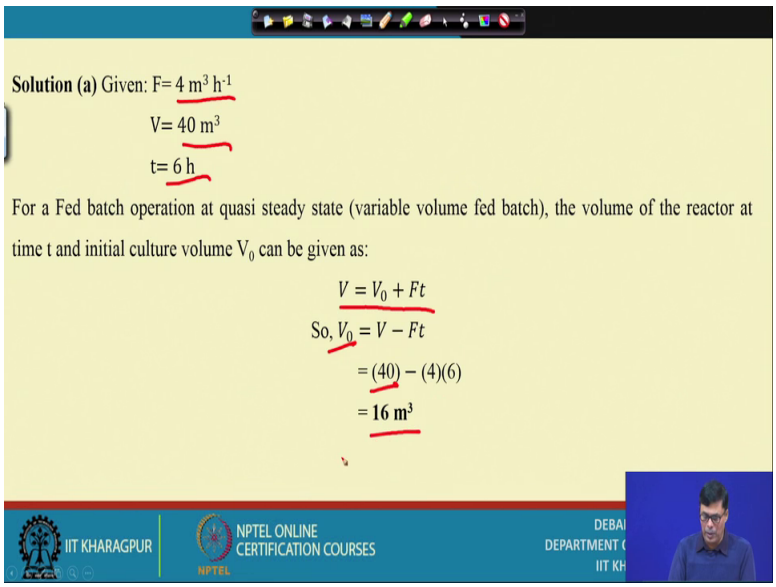

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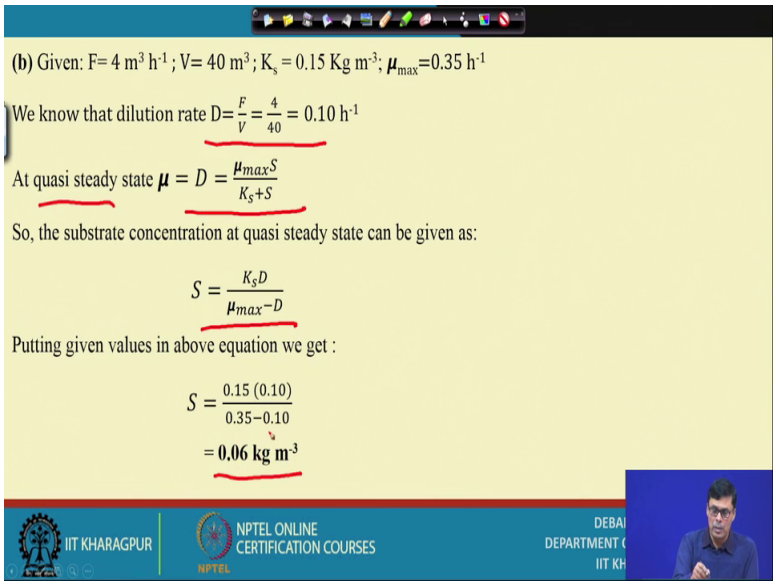

Now, in the next part, that you can find out the dilution rate dilution is nothing but, flow rate by volume this is 0.1-hour inverse mu equal to D under quasi steady state condition and then S values this we can put the different values in this equation, then we can find out the steady state substrate concentration.

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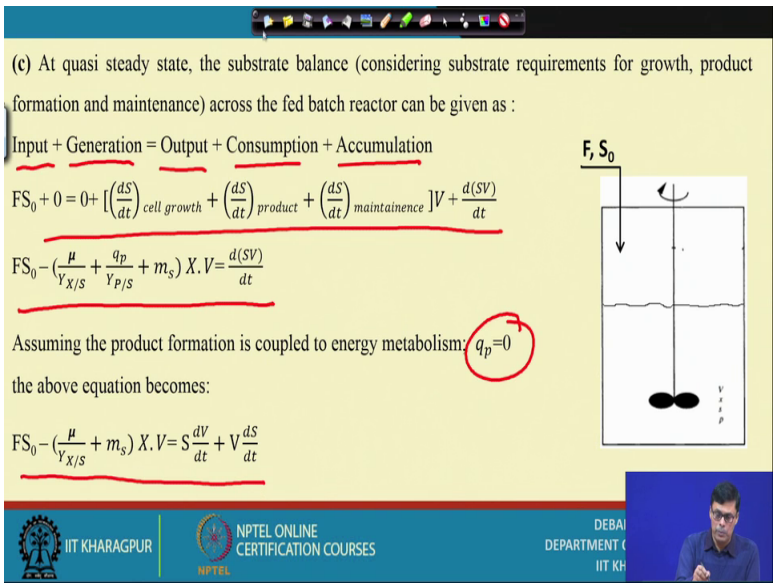

Now, once you know that, then next step is that we shall have to do the substrate balance across the reactor equation is rate of input plus rate of generation rate of output rate of consumption plus accommodation. So, we can write whole the equation like this and this

is the final equation we will be having and if we consider qp equal to here, we do not consider other than cell mass as a product. So, we can assume qp the product specific rate of product formation equal to 0, then our final equation will be this. This equation, if we further analyze you will get that this equation and then, a steady state condition that, rate of the accumulation of the substrate that should be equal to 0.

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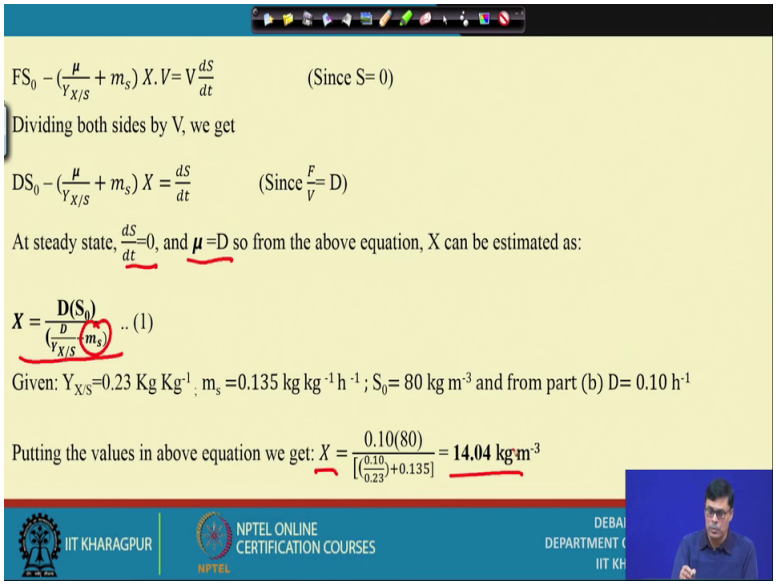

So, this is the exactly what is written there, and mu equal to D that is, then we can write this equation in this form this is the X equal to DS 0 by D YX by S by m s, m s is the maintenance coefficient and if you put the value you can get the final cell mass concentration.

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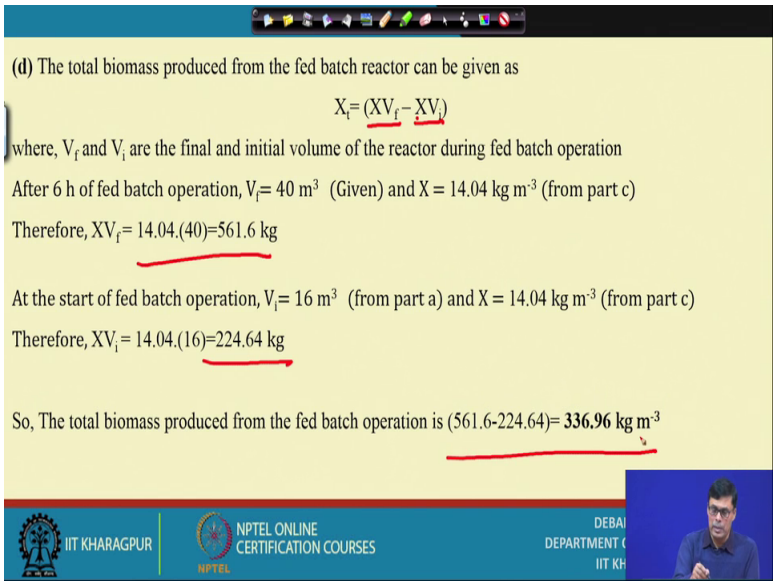

And then, the total biomass will be what total bio mass is the X, X is the cell mass concentration with the final volume and x is the cell mass cell mass concentration in the initial volume.

So, we can find out this value, final cell mass concentrate will be amount is 561.6 kg and the initial will be this. So, the difference will give you the total biomass, that is produced in the system you know that, so this particular lecture I tried to covered several problem 4 different problem and I hope or the batch and fed batch process you can analyze very nicely and you can solve the problem with respect to substrate conversion, with respect to cell mass formation, that we can easily monitor.

Thank you very much.