Aspects of Biochemical Engineering
Prof. Debabrata Das
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture - 39
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – IX

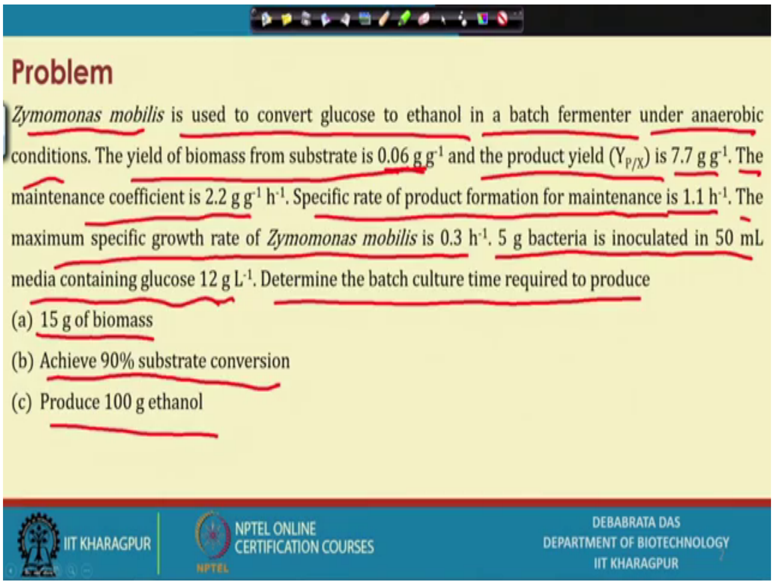
Welcome back to my course Aspects of Biochemical Engineering, and we are last couple of lectures we try to discuss some problems that associated with this cell growth kinetics. We try to solve several that numerical problems and we discussed that initially we discussed the batch process, then how you can monitor the cell mass concentration and substrate concentration and different times.

Then I try to discuss that the fed batch process, how you can find out the cell mass concentration, how we can increase the cell mass concentration in the fed batch reactor. And then in the last lecture, I did some detail analysis on the chemostat process because chemostat is appears to be the most advance process as well biochemical industries are concerned.

Now, this lecture we want to because last lecture if you look into that mostly we give a gave stress on the substrate conversion with respect to cell mass formation and maybe other than cell mass that is the product formation, but here we also considered as a that very interesting term what you call maintenance of the cells, that you know that I told you that part equation, that deals with the maintenance of the cells that you know when cells we keep it in a particular culture, and they move one place to others, they require some kind of energy not only for the cell when cells undergo some kind of ruptured for the repairing of the cells we required some kind of energy. So, all this that the how the maintenance energies use.

So, these problems will deals with that part let us see how we can solve this problem by using all the three factors. Now we have come across three factors one is your cell mass formation, your product formation and the maintenance of this is. So, substrate is going for all the three purpose.

(Refer Slide Time: 02:30)



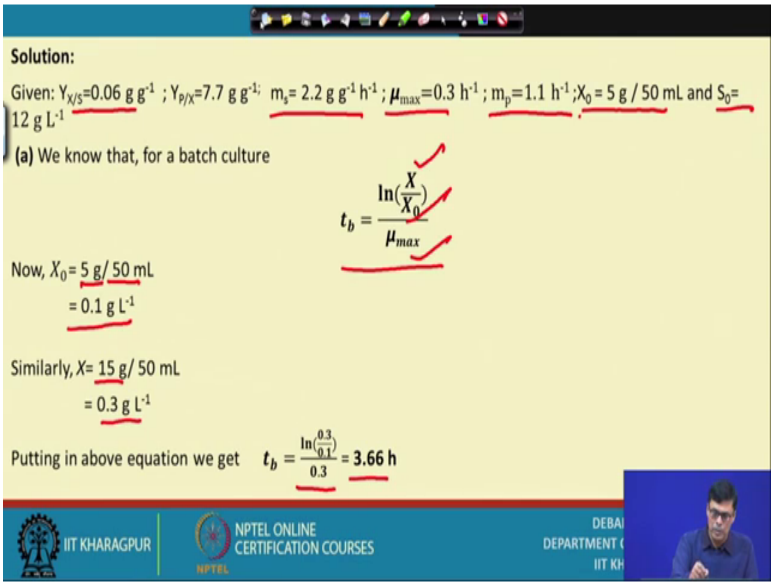
Now, the first problem if we look into this is the Zymomonas mobilis, I told you Zymomonas mobilis is kind of bacteria. Now mostly the alcohol ethanol fermentation take place by using each cells the saccharomyces service here. Now scientists they are working very hard how this the Zymomonas mobilis can be used for ethanol fermentation, because doubling time of Zymomonas mobilis is much less as compared to the each cells. Now this is Zymomonas mobilis is used to convert the glucose to ethanol in a batch fermented under the anaerobic conditions.

The yield of biomass from the substrate is 0.06 gram per liter. When the here I want to just point out that if we look at the aerobic conditions the yield of biomass is very high maybe 0.3 to 0.5 grams per grams, but anaerobic condition it is a usually we have very less amount of cell mass formation that is the here it is 0.06 gram per gram per gram.

The productivity product yield the Y p by x equal to 7.7 gram per gram; that means, 7.7 gram of ethanol produce per gram of p cells. So, gram of bacterial cells. The maintenance coefficient is 2.2 gram per gram per hour. That and specific rate of product formation for maintenance is 1.1 hour inverse and the mixed maximum specific growth rate of Zymomonas mobilis is 0.3 hour inverse and 5 grams of bacteria is inoculated in 50 milliliter media containing glucose, 12 point gram per liter. Determine the batch culture time required to produce, 15 grams biomass achieved the 90 percent substrate conversion and produce that the 100 grams of ethanol.

So, you know that the problem has 3 different parts, one is you have to find out to get the 15 grams per liter grams of biomass how much time is required in a batch process, then you have to find out that to achieve the 90 percent substrate conversion, how much is a that is time is required, then to produce a 100 come of ethanol that how much time is required. So, this I consider this is a very interesting problem let us see how we can solve this problem.

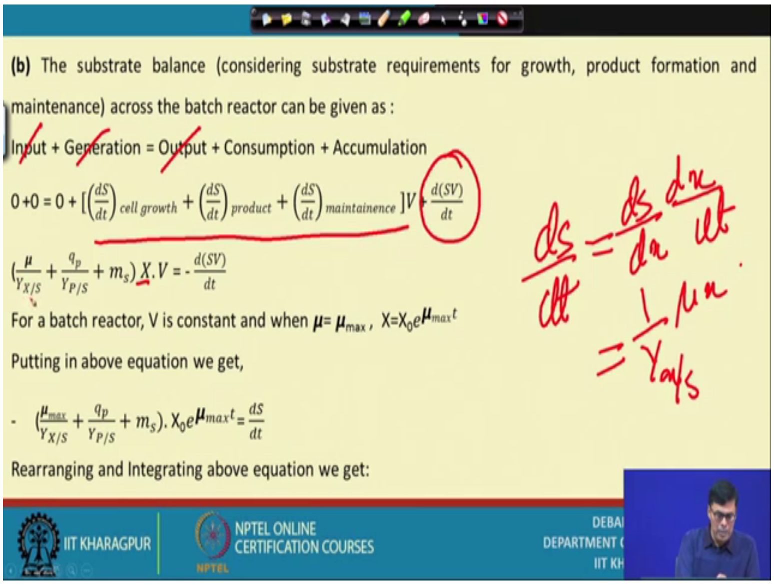
(Refer Slide Time: 05:19)



Now, as I mentioned before that whenever we want to solve any problem, it is better to write what are the given data is given, what are the given data Y x by s. So, that is what we call cell mass yield is the 0.06 gram per gram, Y p by x 0.77 gram per liter, maintenance coefficient is given, mu max is given mp the materials for the product formation, x 0 is given 0.5 gram per 50 milli milliliter and S 0 is 12 gram.

Now, we know that in the batch culture this already we derive equal to ln X by X 0 by mu max. Now X 0 value we can easily calculate the 5 gram over the initial cell mass concentration your culture volume is 50 milliliter. So, you cell mass concentration is coming around 0.1 gram per liter am I right and final cell mass concentration is 50 gram. So, divided by 50 that is 0.3 gram per liter. So, here in this problem we have X value we have X 0 value, we have mu max value. So, if we put all this value we can find out what is the time required for the batch process.

(Refer Slide Time: 06:43)



Now, if you look at the second part, that you know this is the first part is to how what is the time required to get 50 grams of biomass, second part is that the what is the time required to get the 90 percent substrate conversion, let us see how we can solve it.

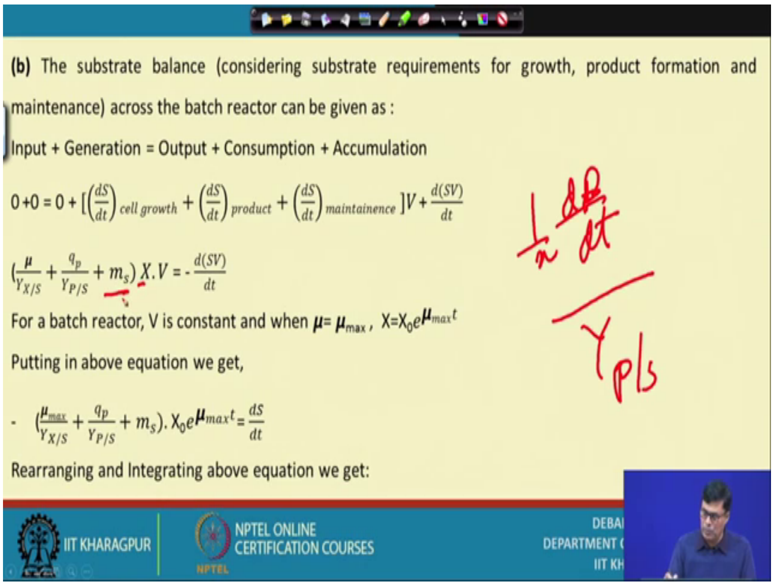
Now, for doing so, first we shall have to do the substrate balance. Now what is the substrate balance? Rate of input equal to rate of generation plus rate of output rate of consumption and accumulation. Now since this is a batch process rate of input and rate of output that should be equal to 0 and generation also equal to 0 am I right. So, what will be having that we have the rate of substrate growth, and rate of product formation and maintenance into V, that the as I pointed out before we considered only the cell mass growth am I right.

Now, we are considering three portion, that is the substrate is used for growth of the cells one part is going for the product formation, there here is the ethanol another part is the utilizing for the maintenance of the cells. So, you have to consider all the three part and then this is the accommodation of the substrate.

So, this say this I can write like this that what is the dS by dt for cell growth how we can calculate that is I told you that dS by dt is equal to what? This is dS by dt I can easily write that dS by dx into dx by dt I showed you before also this is just repeating this is 1 x by s and this dS by dt is mu into x. X I have taken common. So, mu into x divided by y x by s that will give you the substrate equal for the cell growth.

Now, similarly for the product we can also substrate use for the product, I can write a similar way with the dx by d p into dP by dt I can write this. So, we that you know that with that pq what is q p? Q p is the specific product formation rate.

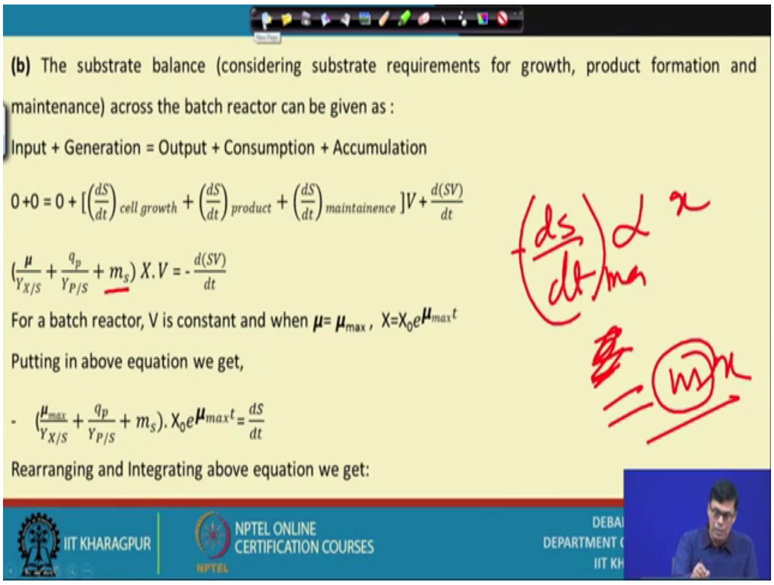
(Refer Slide Time: 09:06)



What is specific product formation rate? 1 by x dP by dt am I right dp by dt. Now dP by dt now divided by Yp by s; Y yp by s equal to dp by ds the dp by dp will be dp will cancels the dS by dt the and if you multiplied by x; then, will get the substrate required for the product formation.

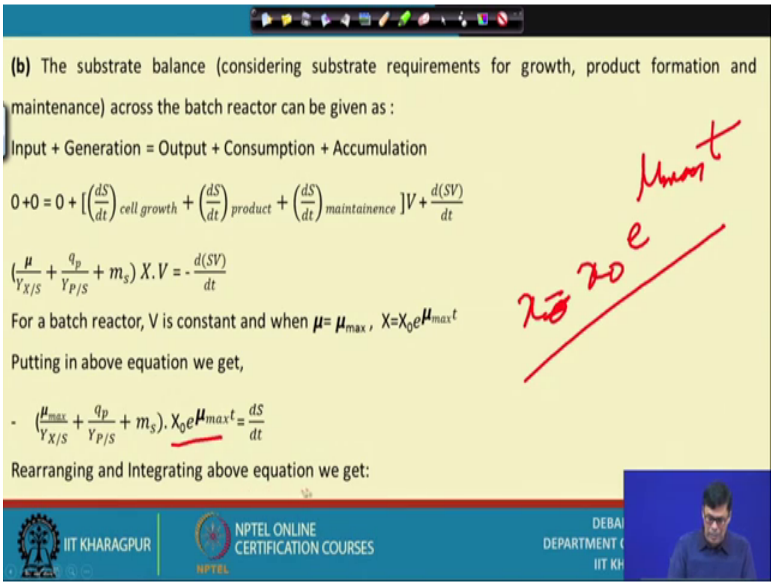
Similarly, we can find out the substrate required for the maintenance of the cell, if you multiplied by x we will get the substrate required for the.

(Refer Slide Time: 09:43)



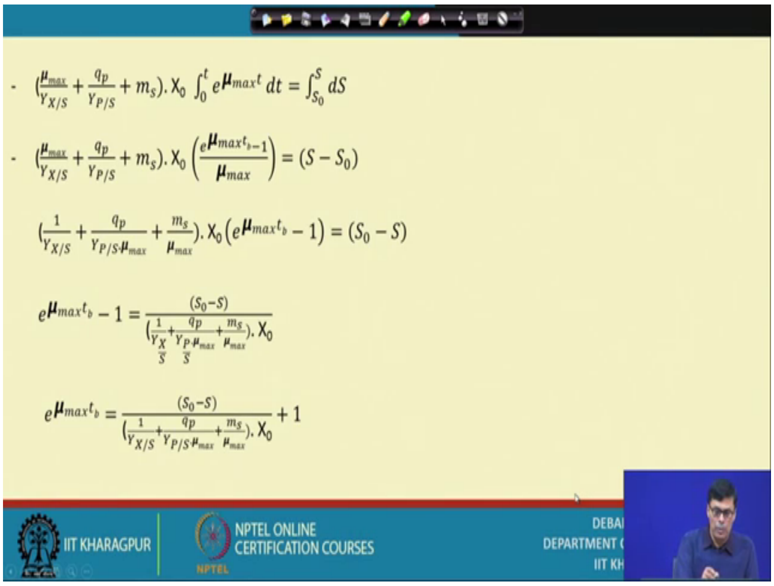
Because we have we know that a rate of substrate that is utilized for the maintenance of the cells is proportional to what cell mass concentration. So, rate of substrate required for the maintenance will be equal to what? Equal equa to m into x, the x is the for the kind of constant what do you called my Michaelis Menten what? Do you call maintenance coefficient this is a maintenance coefficient. So, this equation; obviously, it has come.

(Refer Slide Time: 10:12)



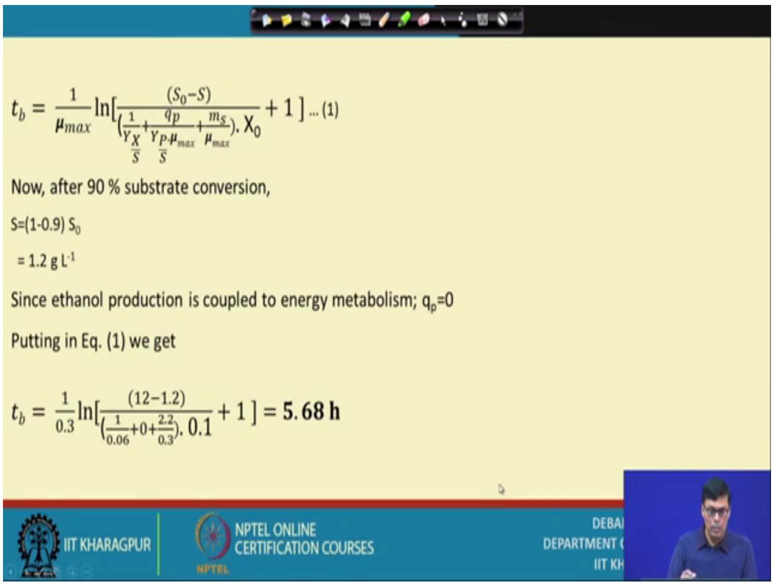
And we have already calculated what is the x value how; you can calculate x equal to x 0 e to the power mu max into t I we have shown you before I am not showing you again here. So, this is what we can put it here and then this is equal to dS by dt by rearranging the above equation we get then we will we will get this equation final equation will get this equation the rearranging of this equation.

(Refer Slide Time: 10:36)



Then we solve this equation finally, we will come in this equation e to the power mu max into t b equal to S 0 minus S 1 by Y x by s qp yp by s mu max may ms by mu max into x 0 plus 1 this is we can find finally, but you can I hope you can solve by yourself and find this equation.

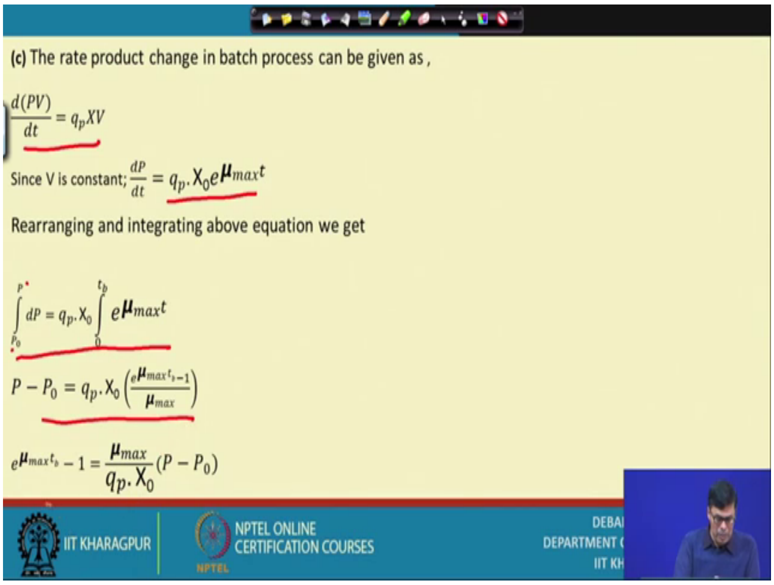
(Refer Slide Time: 11:13)



Now, then from that we can if we look at this equation is the this a I can now you divide by tb you can easily calculate, you can tb equal to 1 by mu max, that ln this value. Then for 90 percent of substrate conversion, that what is the is way is the value that the t10 percent will remain that one gram per that if you see that what is the s value is given 20 gram per liter am I right twelve gram per liter.

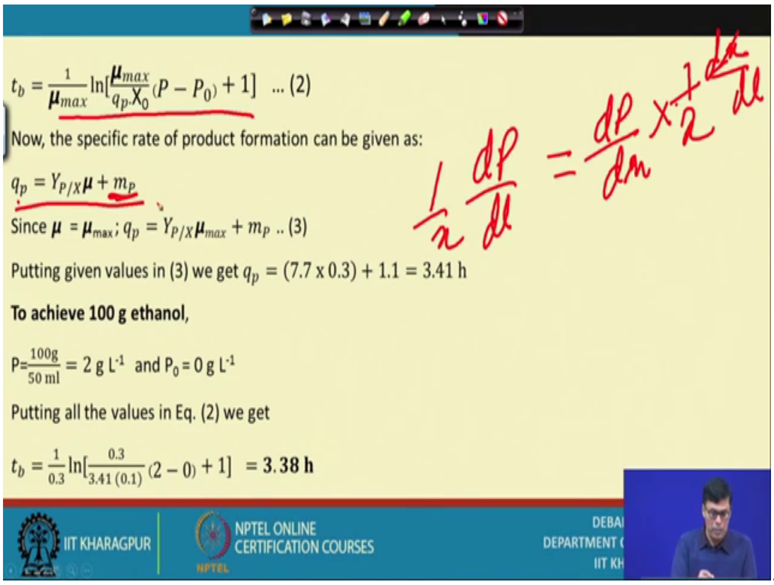
So, here we have done little mistake this is no mistake because this is this is actually that S 0 is 12 am I right and this is equal to 0.5. So, you multiply that one point two this is this is not a mistake and then we put the value different values are given in this problem and qp I can assume there to be constant because you know in this problem if we assume this is equal to cell mass growth and when, there is a in this equation you have three part am I right when you have you have this is the cell mass growth and another is the product formation and the maintenance. Since ethanol it produces coupled with energy metabolism then we considered qp equal to equal to 0 and in that case that tb will be equal to 5.68 hours.

(Refer Slide Time: 13:35)



Now, rate of product for change v formation can be written as the d PV by dt equal to qp into X into V is constant, that dP by dt this we can write this is the x x. So, again we can write in this form into v we can V; will cancel am I right and then we rearranging this equation we will find this equation and dP will be integrating from p 0 to p.

(Refer Slide Time: 14:13)

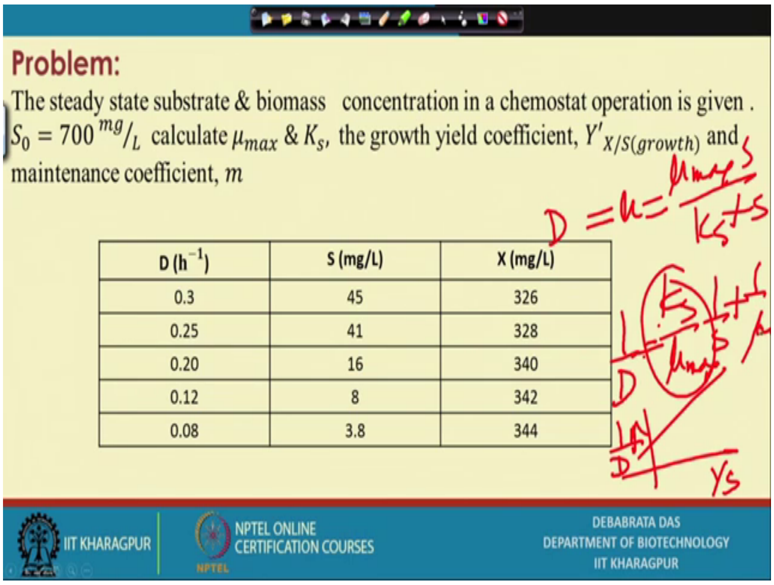


And this will be the final equation and we will get this equation in this form. Then t b equal to coming in this form tb equal to this form this is coming then .

Now, the specific rate of product formation can be this qp equal to this is this form then Y p by x into mu max because I can show you that dp this is dP by dt this specific rate of equal to this is what Y p by x y p by x means dp by dx am I right dp by dx and this is mu equal to 1 by x dx by dt.

So, the this will cancel. So, we can write specific rate of product formation and this is the maintenance that is required during the this product formation. So, we can write this is the specific rate of product formation is like this, and then we have this equation and putting these values we can we will get the qp equal to 3.4 one hours to trichive to achieve the 100 gram of this ethanol the p equal to what 500 divided by 50 milliliter is culture the concentration will be two gram per liter p 0 equal to 0 then tb equal to I can put this equation in this equation if we put is this coming around 3.38 hours. So, I after 3.38 hours you will get the 100 grams of ethanol. So, although all the three problem problems you can solve.

(Refer Slide Time: 15:48)



Now, in the next equation is little bit simpler, I hope you understand this the steady state substrate and biomass concentration in a chemostat is given S 0 is this and calculate the mu max and K s and growth yield coefficient and this is the true will coefficient Y dash x by s and the maintenance coefficient. Now up till now we did not discuss that how to determine the true growth yield now, what is true grow yield? We up till now we come across the overall growth yield. Overall growth yield means gram of cell produce per gram of substrate consumed.

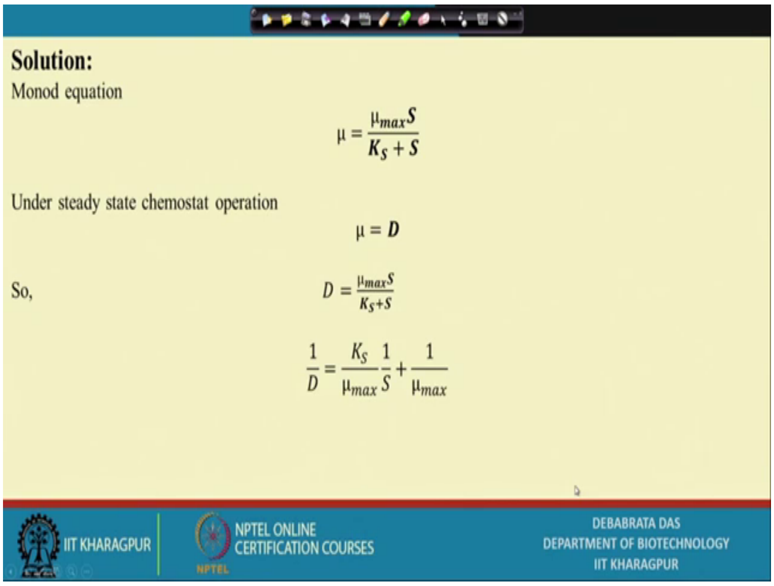
Now, when we say gram of cell produced it may compresses the both the vivel cells and the dead cell, but you know that when you or you know that, but you know that amount of cell mass that is produced that during that a part also go for the maintenance of the cells. And also a part goes for the product formation also so, but a when you say that we will consider the true growth yield then the product only goes for the cell mass formation because the how much the when that this is not used for maintenance or product formation that is called true growth yield.

So, question comes how you can calculate the true growth yield, and the maintenance coefficient on the this we have not discussed yet then let us see how we can solve this, and another very into interesting things that, up till now the cell growth kinetics we try to estimate by using the batch process. Batch process we find out that at the first problem we solved that you know at different times we have cell mass concentration, we have substrate concentration then we from that we find out the mu value, we find out the 1by mu and 1 by s value we plot the lineweaver Burk plot and we determine that the ks and mu max value.

Now, same the question comes the is it possible to estimate the kinetic constant by using chemostat answer is yes, but now how it can be done? Because you know that a Monod equation we know mu equal to mu max S Ks plus S am I right. Now under steady state condition and sterile feed mu equal to D. Now if a mu equal to D, I can write in the lineweaver Burk plot this is 1 by d equal to Ks by mu max 1 by s plus 1 by mu max am I right.

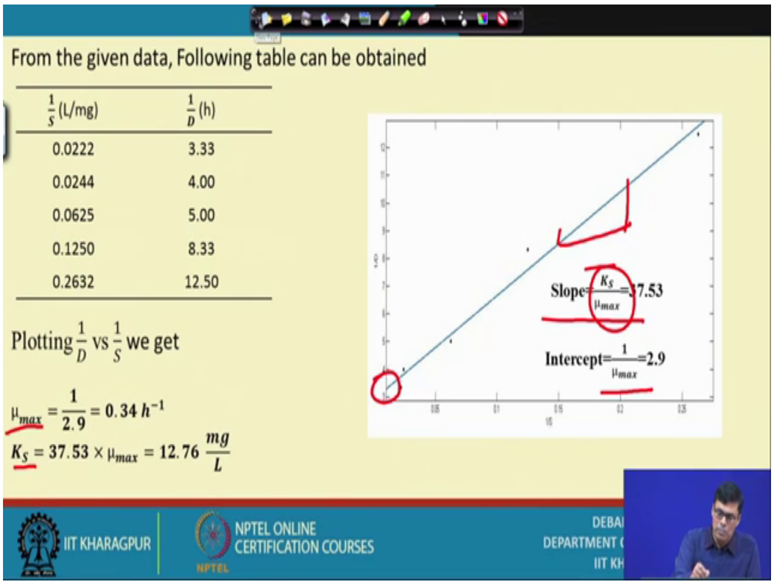
So, now you can you can easily plot 1 by in case of 1 by mu, I can plot 1 by D versus 1 by S. So, you can have a straight line we from the slope we can find out a ks by mu max and for intercept we can 1 by mu max.

(Refer Slide Time: 18:48)



So, this we can easily find it out. Now this is exactly what we is done here this is this is the Monod equation that we have an under sterile condition and steady state mu equal to d then we can write in this form.

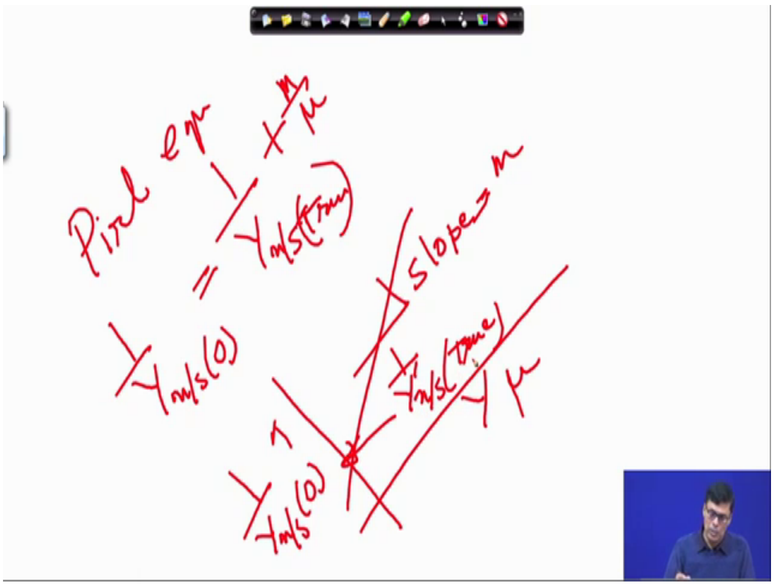
(Refer Slide Time: 19:02)



Then we have we have 1 by lth value 1 by mu value we can take in a graph paper simple centimeter graph paper, we can plot this and from the intercept; from the intercept we can find out the value of 1 xby mu max and from the slope we can from the slope we can find out the value of S by mu max and then you can find out both the value of mu max and K s we can calculate from this from this plot.

Now, question comes how you can determine the maintenance coefficient because you know that is that is the important.

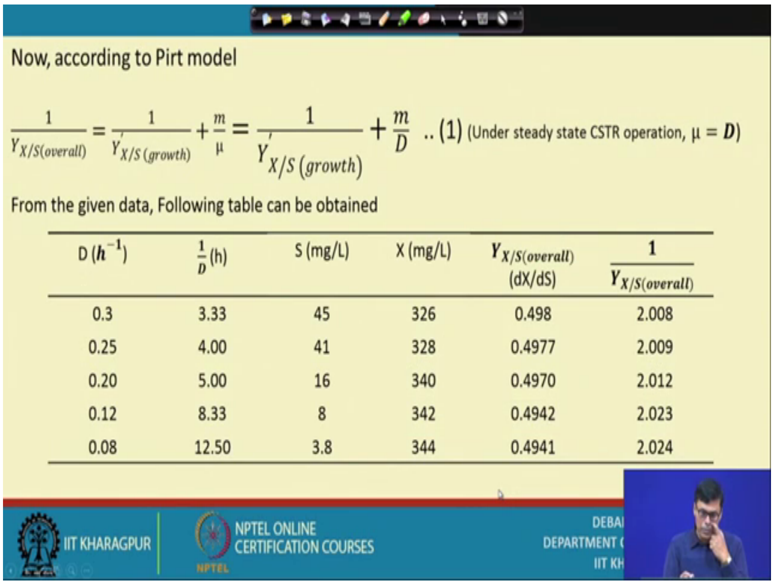
(Refer Slide Time: 19:48)



So, for maintenance coefficient if we will know at the Pirt equation, what is the Pirt equation we have seen? That is 1 by Y x by s this is overall am I right this is equal to 1 by Y x by s it is true growth yield true; true growth yield means I i told you that when substrate is used for only for the cell mass formation this is into m by mu am I right.

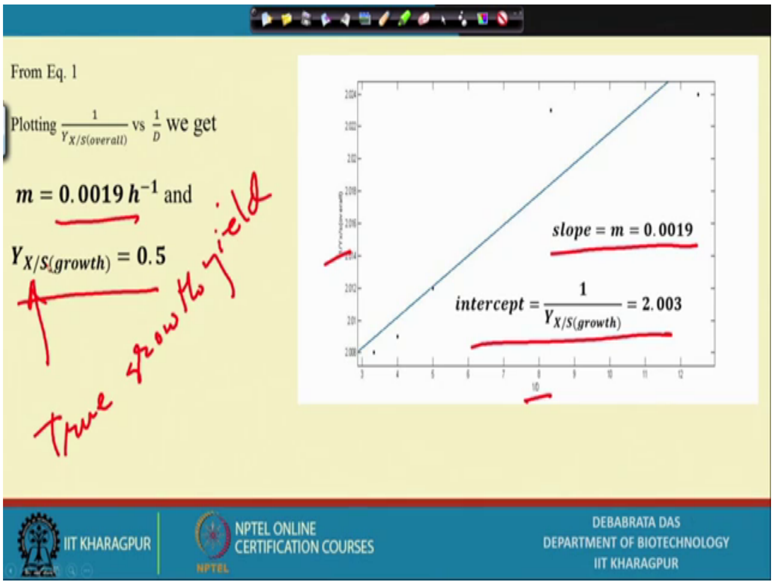
Now, if you plot Y x by s overall this is 1 by mu, then you will get a straight line slope will give you the value m am I right. And this intercept will give you the value of Y x by s true growth yield you can easily find out what you call we sometimes we considered as the Yx by deaths y x by s the deaths this is true growth yield. So, we can find out these values.

(Refer Slide Time: 21:03)



Now this equation exactly we have written in this form, this is the 1 by x this is like this and this mu we replace by D, and then t we can have this table, we can write this table from this data that we have from the we can data we can write this table.

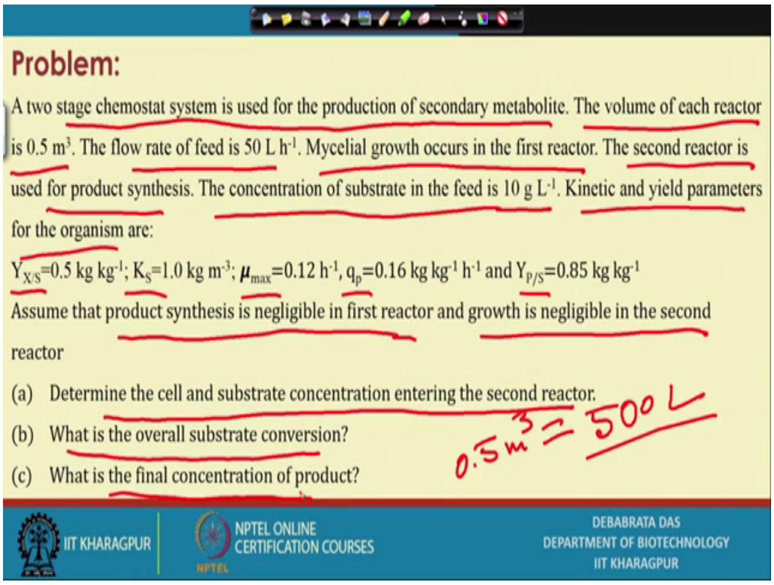
(Refer Slide Time: 21:41)



And then we can plot here we have the plot of 1 by y by x versus 1 by d, if we plot it then we will get a this intercept slope will give you the value of maintenance coefficient and intercept will give you this.

The maintenance coefficient will be 0.0019 hour inverse and yield coefficient will be 0.25 this we can easily and this yield coefficient this is called growth yield growth yield is what you call true growth yield whens the substrate is used only for cell mass group. Now it is not used for neither materials nor the product formation then we call it true growth yield and this remains always constant for a particular organism they should remain constant.

(Refer Slide Time: 22:36)

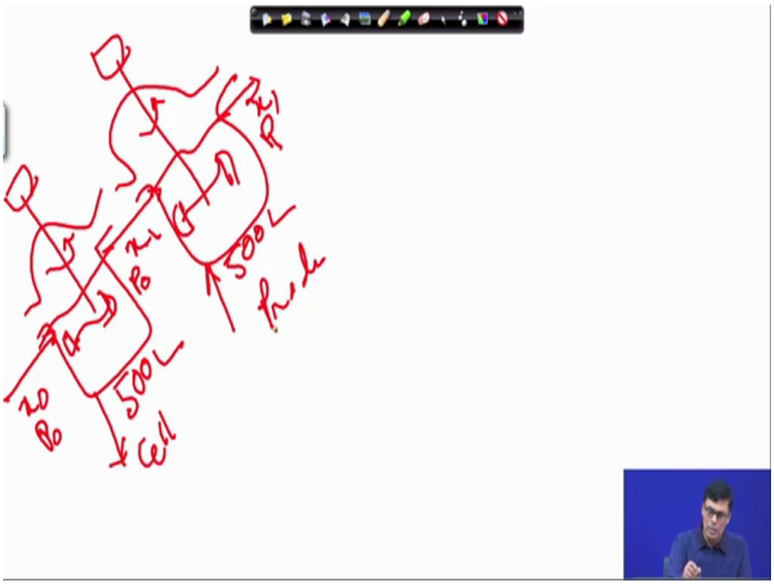


Now, next problem I hold I i hope that this problem is the typical problem that to k chemostat system is used for producing the secondary metabolites, we know the secondary metabolites that usually it is most of the secondary metabolized form during the stationary base, you can you know that and the volume of the each reactor is 0.5 cubic meter 0.5 cubic meter means how much? 0.5 cubic meter means how much this is one cubic meter is 1000 meter the 1.5 is equal to 500 liter am I right .

So, the flow rate is 50 liter per hour, the mycelial growth occur in the first reactor and second reactor is the used for the products synthesis. The concentration of the substrate in the feed is 10 gram per liter, the kinetics and yield parameters of the organisms and Y by s Ks mu max q p and Y p by s is given here. Assume the products synthesis is negligible in the first reactor growth is negligible in the second reactor, determine the cell and substrate concentration entering in the second reactor what is the overall substrate conversion what is the final concentration of product

So, what is the problem because you know let me explain the problem. So, that you know the problem is like this.

(Refer Slide Time: 24:11)

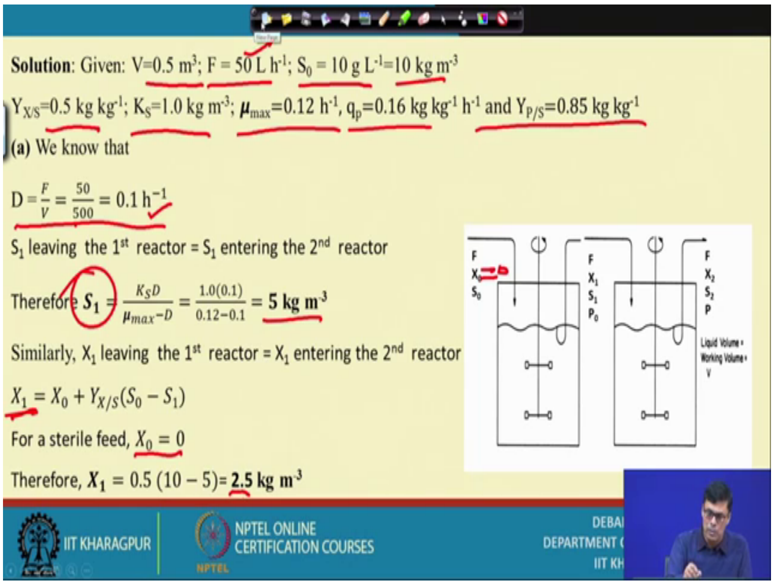


We have two reactor this is one and this is two this is like this. So, we have this starrer here and here we have a starrer am I right and this is liquid and this is coming like this and this is the starrer, and here this volume is 500 liter and this is also 500 liter both capacities 500.

Now, here only the cell formation is there, and here only the product formation is there that is the assumption we have. So, we can assume here cell formation. So, here this is the x 0 and this is x. So, whatever x is there or x one and here also it is x one because we assume that a no cell mass formation is there, and here also that is the you know here p p 0 is this is also p 0. So, here will be p one only product formation is there.

So, this is the how this problem that will we have we shall have to dealt with .

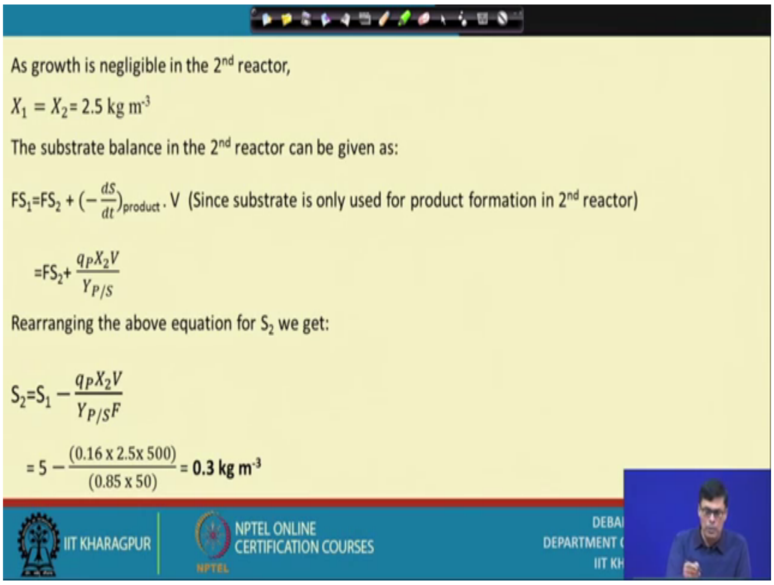
(Refer Slide Time: 25:31)



So, what are the parameters are given what was the parameters are given this volume is 0.5 cubic meter flow rate is 50 meter per hour s 0 is t10 gram per liter ten gram per liter we can write ten kg per cubic meter and Yx by s is 0.5 kg per kg ks is 1 kg per cubic meter mu max is 0.12 qp is given and this is given.

Now, what is the dilution rate I can write, this is the flow rate 50 liter per and 500 milliliter. So, 0.1x is the dilution rate from that we can easily find out S 1 value s one value is equal to Ks into d divided by mu max minus D. So, this is five kg per cubic meter similarly we can find out the cell mass concentration if we assume x 0 equal to 0, this equal to 0 if we assume then we can find out the x 1 value it is coming about 2.5kg per cubic meter am I right.

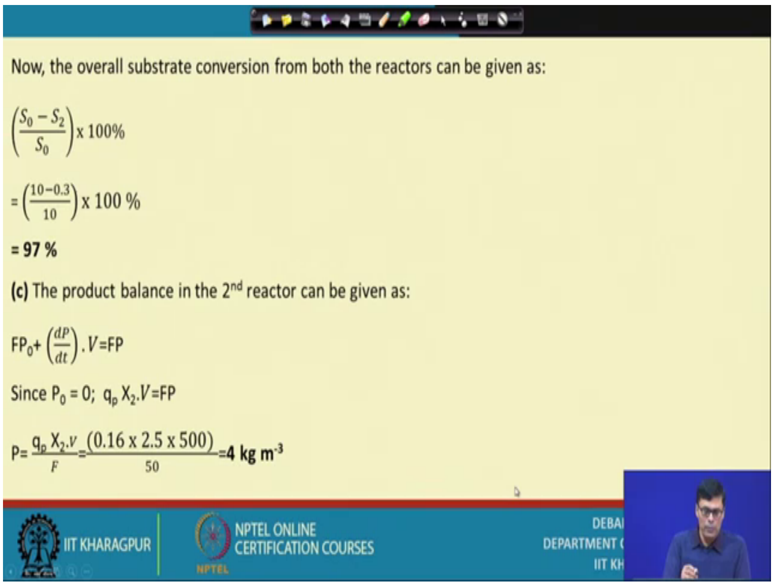
(Refer Slide Time: 26:28)



Now, the. So, in this problem the second reactor it is assumed that no cell growth, I can write X 1, X 1equal to x 2, that is 2.5 kg per cubic meter. The substrate values in the second reactor how we can write the rate of substrate input my f into s one and rate of generation will be 0 then rate of substrate output f into s 0 rate of substrate consumption in to volume.

So, this we can write in this form that the rate of product formation, and then we can write the s 2 value S 2value is coming around 3.3 gram kg per cubic meter.

(Refer Slide Time: 27:10)



So, what will be the final overall conversion of the process? Initially we have ten gram per liter finally, it is coming 0.3 10 minus point three by ten that is 97 percent.

Now, that is the next post performance that what is the final product concentration the product balance in the second reactor is F nto P 0 dP by dt in the p into fp am I right. Now if it is like this then the p 0 we can assume to be 0 and then this equal to this and this all values are given the flow rate and qp X 2 and v value is given. So, we can easily find out final product concentration it is not very difficult.

So, in this particular lecture what I try to analyze, I try to analyze that in a particular bio biochemical process that you know three things simultaneously take place, when substrate is consuming that your cell mass formation is there your product formation also there and maintenance of the cells. Now when you when all the three things are involved in this process how we can take care to find out the different parameters in the system.

So, in the previous lecture we showed you how we can find out the kinetic constant like mu max and Ks value. Now in this particular lecture I try to tell you not only Ks and mu max value in the, but also how you can calculate the value of true grow yield also maintenance coefficient, and also while you try to find out that how you can calculate the product for a concentration in the particular reactor final product concentration how you can find out.

Thank you very much.