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

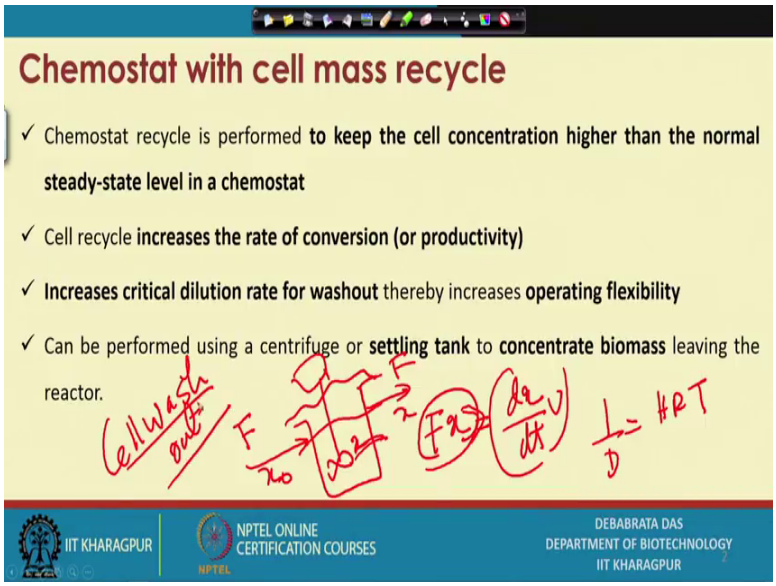
Lecture - 34
Kinetics of Substrate Utilization, Product formation and Biomass Production of Microbial Cells – IV

Welcome back to my lecture that Aspects of Biochemical Engineering. Now, last couple of lecture, I try to concentrate on cell growth kinetics that we discussed the cell growth kinetics both for the best system and the continuous system. And when we discuss the chemostat process, I told you that, we know the continuous stirred tank reactor. When continuous stirred tank reactor we use in the biological system, this is we call it chemostat.

And major drawback of the chemostat process is the cell mass that is going out of the system. Now, if the rate of cell mass that is going out of the reactor, is more as compared to rate of a cell mass that is growing in the system, then what will happen is, situation will come when there will be no cell present in the reactor. Now that situation, we can overcome by using 2 different approaches.

One is called cell mass recycling, that another we called immobilized whole cell. Now, there, so today, I shall concentrate on that you know how with the help of cell mass recycling, we can control the cell mass waste in your wasting in your from the system or the wash out of the cells.

(Refer Slide Time: 01:55)



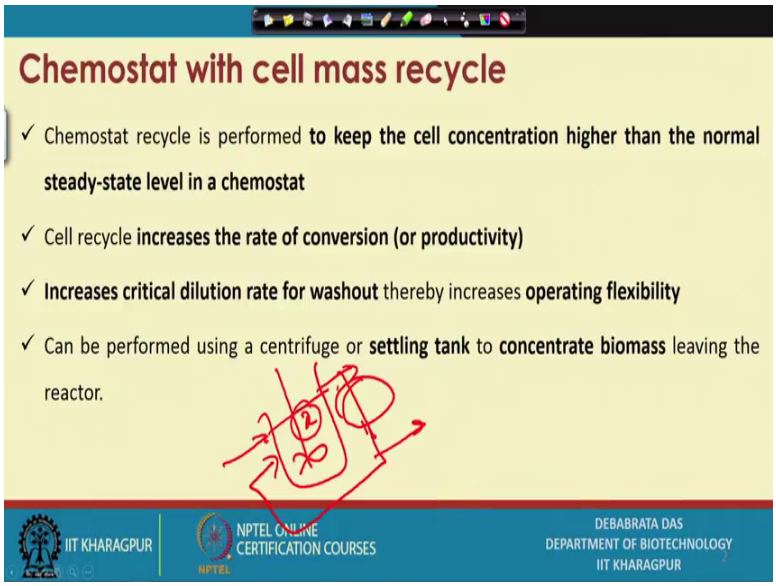
Now, first, you know, let me explain this, because, suppose this is C S T R, and this will continuously liquid is coming at a particular flow rate and going out at the particular flow rate.

Now, here x 0 this is x and this is also x. Now what I want to point out that, always you will be having cell wastage, what is called F into x, am I right? So, if this is equal to rate of growth of the cells inside the cell, inside the reactor, then it is fine. Because, if it is the growth, say that, you know that if the rate of growth is same as that, then it is fine. Otherwise, if it is more than that, then the cell concentration will decrease with respect to time. A time will come when there is no cell present in the reactor.

Now, another explanation to this process I explained that is the generation time; because, 1 by D is considered as the hydraulic retention time. Now, what is hydraulic retention time means, how long you allow a particular liquid research in the reactor. Now if you have generation time is more than that hydraulic retention time, then what will happen that your liquid will get less time. So, naturally your cells will not grow inside the reactor and before it grows, you are taking out the liquid from the reactor. That is why, we phase the situation what we call cell wash out.

Wash out means, the after some time, you will find there is no cell present inside the reactor. This is the major drawback of the chemostat process.

(Refer Slide Time: 03:49)

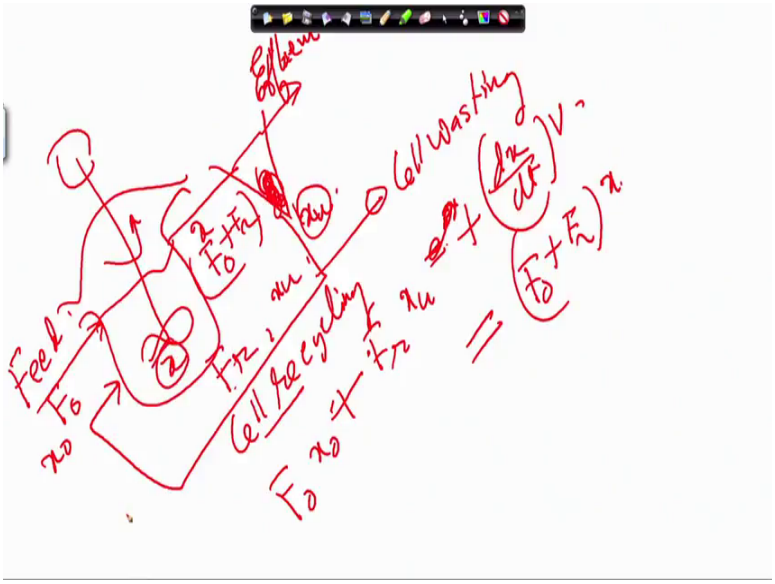


Now, this can be overcome. How you can overcome? This can be overcome, suppose, the whatever x s s cell mass is going, so, if you put a separator here and we can and part of the cell, you are recycling back to the system to maintain the cell mass concentration uniform, then and all then your rate of reaction will be constant. Then there will not be any problem of cell washout. So, this we will discuss here.

The chemostat, we sell must recycling the chemostat recycling is performed to keep the cell higher than the normal steady state level in make chemostat. So, we recycle in the manner, so that, you cell mass concentration it remain constant higher than that. So, we ensured that, your rate of reaction is constant or more than that, we have we desire that is, cell recycling increases with the rate of conversion. And obviously, that increases in a critical dilution rate for wash out there, but increases the operating flexibility.

And can be performed using the centrifuge or settling tank or to concentrate biomass leaving the reactor.

(Refer Slide Time: 05:11)

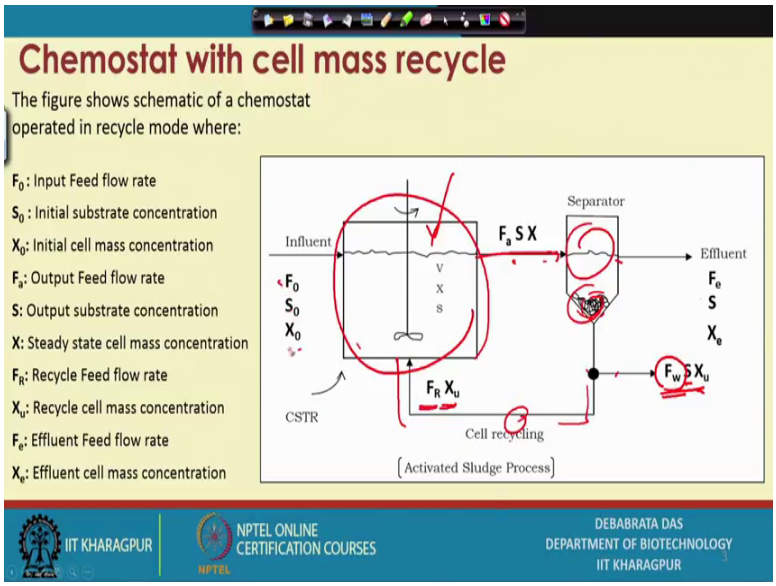


So, if we see that you know that C S T R of the cell mass recycling system, it basically looks like this. So, this is rotation; this is like this. So, here we can have the settler and this is going out and part of the things you recycle back and part use. So, here what you call cell recycling, this is recycling. And this is called cell wasting.

And, this is actually the effluent; there is a clear liquid effluent. This is the feed we have. Now, suppose, this is the settle cell mass concentration x u and this is x f. When you settle, obviously, the cell mass concentration will be more here; and this is the settle cell mass concentration. And let us assume, the recycle flow rate is F R and this is the F 0. Then, what will be here, flow rate? F 0 plus F R, am I right? So, what is the cell that is going out going in? If we assume this is x 0, so, we can write F 0 into x 0 plus this is recycling; this is also x u.

This is plus F R into x u this is equal to what if a x then, plus there will be some production generation. This is plus the rate of growth of the cells inside the system this is into v this will be equal to F R F 0 plus F R into x 0 x we can write like this. So, this is the main purpose of recycling we want to maintain this cell mass concentration uniform or above certain concentration what we really looking for.

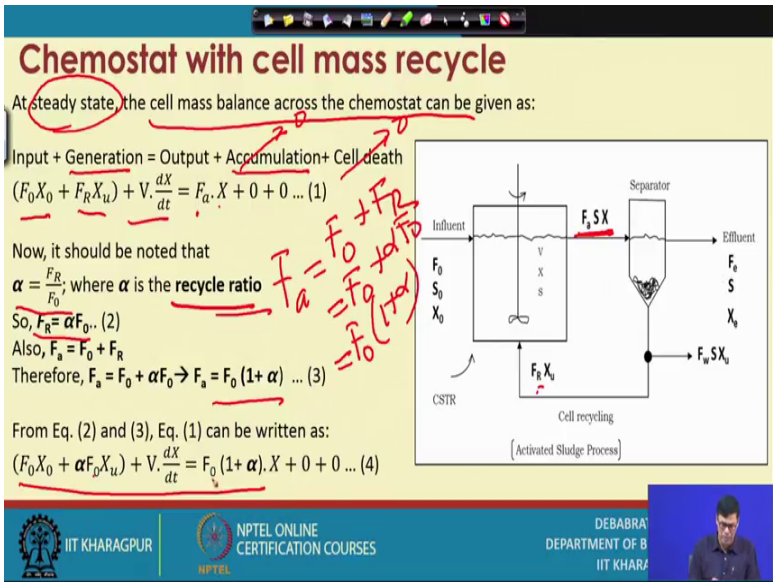
(Refer Slide Time: 07:17)



Now, this is the process how you can be explained in pictorially like this. So, here, as I told you, this is the process, this is the C S T R we have continuous starting reactor or chemostat. And this is the influent and this is what is going out and this is the separator; cell separator concentrated of the cells is there a part you recycle back. This is the recycle flow rate, this is the settlement this is waste recycle flow rate. Yes, we assume here when we carry out the reaction only take place in the reactor; reaction does not take place in the separator of the pipeline. Then we can write the balance equation.

So, here all the parameters are noted here that you know how these are different F 0 S 0 and X 0 and all the parameter what they are stands for.

(Refer Slide Time: 08:26)

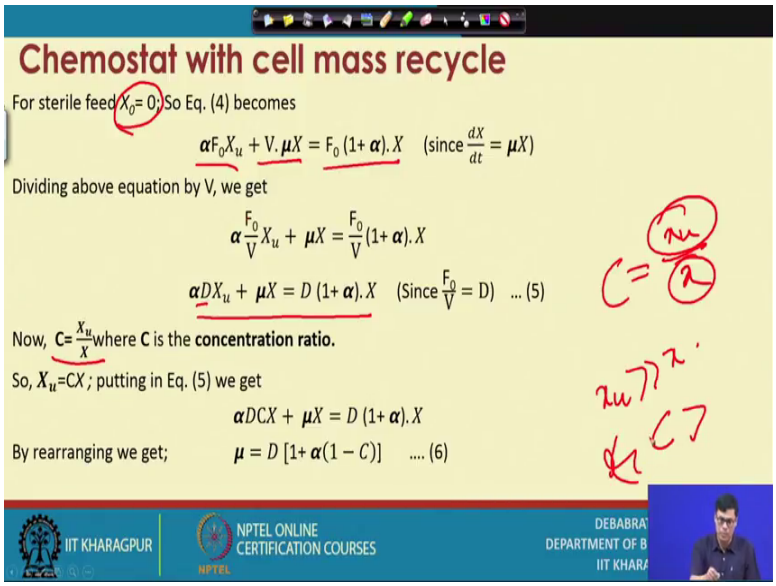


Now, here, question comes, how you can write the balance equation? Let us first consider the cell mass balance across the chemostat. How we can write? I told you what is the input equal to F 0 into X 0 F 1 into X 0 this is the F 1 into X 0 and this is the input to the cell and what is the generation that we have? This is the generation V into X by D t. And what is the output? This is the output, am I right? F a is what F a is equal to F 0 plus F R. So, this is into X and plus accumulation under steady state condition.

So, if we, since we mentioned the steady state condition, we can assume the rate of accumulation is the 0 and also, we can assume that there is no cell death take place. Then what we can write that alpha equal to F R by F 0 and alpha is considered as the recycle ratio. So, what is the F R is equal to what? Alpha into F 0.

Now, F a equal to F 0 plus F R, am I right? Then, F 0 plus alpha into F 0, F R equal to. So, I can call a common F 0. I can write 1 mu plus alpha. This is exactly what is written here. Now, this equation can be modified in this form that, this is a same serve we have the F 0 into X 0 F R is equal to alpha into F 0 into X u and this is like this F 0 this we can modify at F 0 into 1 minus 1 plus alpha into X.

(Refer Slide Time: 10:20)

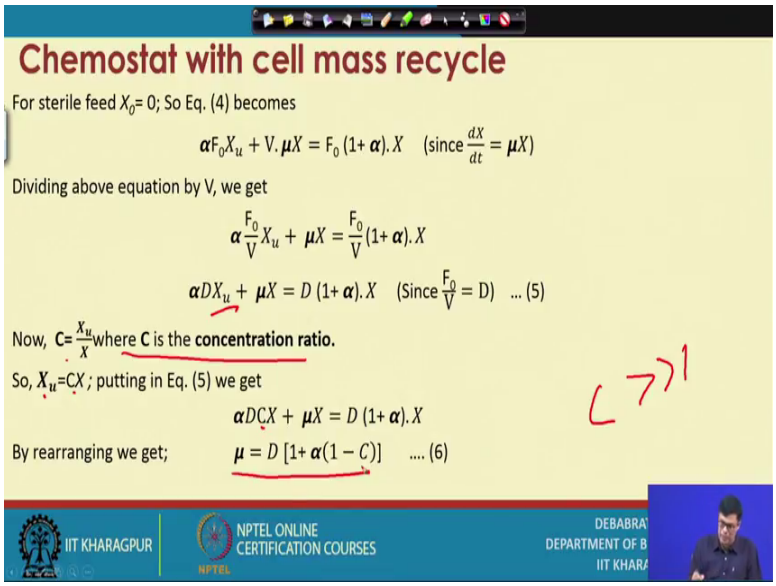


Then what we can write that, we can write in a b b s equation was like this.

And then we can write this is alpha into F 0 this is what we have alpha into F 0 and b into F 0 and because we considered x 0 equal to 0. So, first time we can add that you can neglect and that is equal to this. And this equation, I can write now, we can divide by V both sides and if you divide by F 0 by V equal to what dilution rate. So, this we can modified in this form. Now that now we can assume, C equal to X u by C if you write C equal to X u by X. Now, x x u what settle cell mass concentration.

And X is the cell mass concentration under in the reactor under sterile. So, X u is usually much higher than X. So, I can always say, that C is sorry.

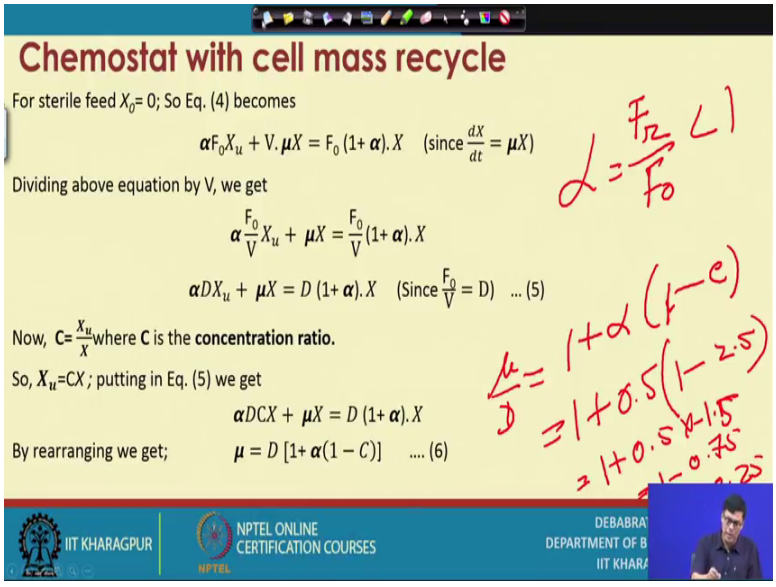
(Refer Slide Time: 11:32)



I can write C always much greater than 1. So, this is like the c equal to the concentration ratio of that. Now we can write X u equal to C into X. Now this we can write in this form then, the X u value how we can put C into X? So, this equation can be modified as like this. Now, what is the significance of this equation? Let us try to find out now here that.

I can write mu by D is equal to 1 plus alpha 1 minus C.

(Refer Slide Time: 12:03)

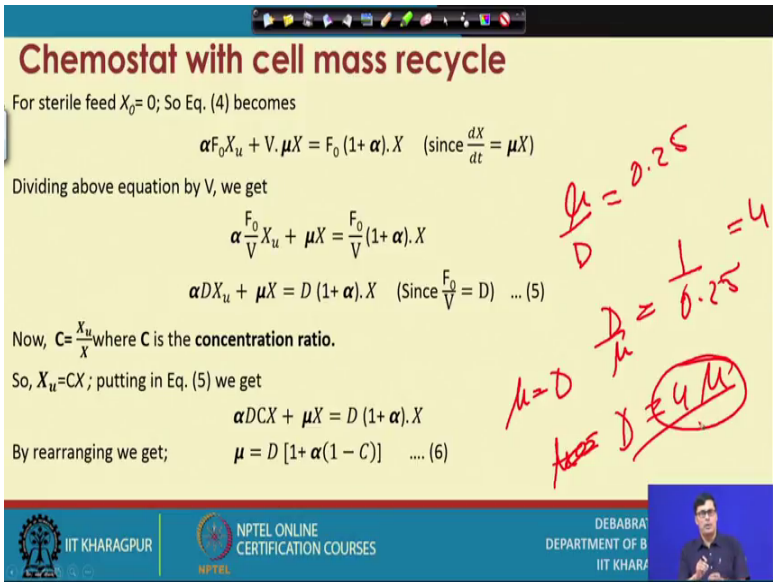


Now what is 1? What is alpha? Alpha is the recycle ratio, alpha equal to what F R F R recycle flow rate by F 0 and this usually less than 1 because, usually the recycle ratio

always will be less than 1. So, let us assume it is of 50 percent 0.5. Let us assume that. And 1 minus and I told you C always should be greater than 1. So, if we assume, this is 2 or you know 2.5 still is assume that then what will happen.

That 1 plus 0.5 into this will be 1 minus 1.5. 1.5 is how much this will be after 1 minus 0.75. So, this will be 0.25. Now, if it is 0.25, so what I can write? The mu by D equal to 0.25.

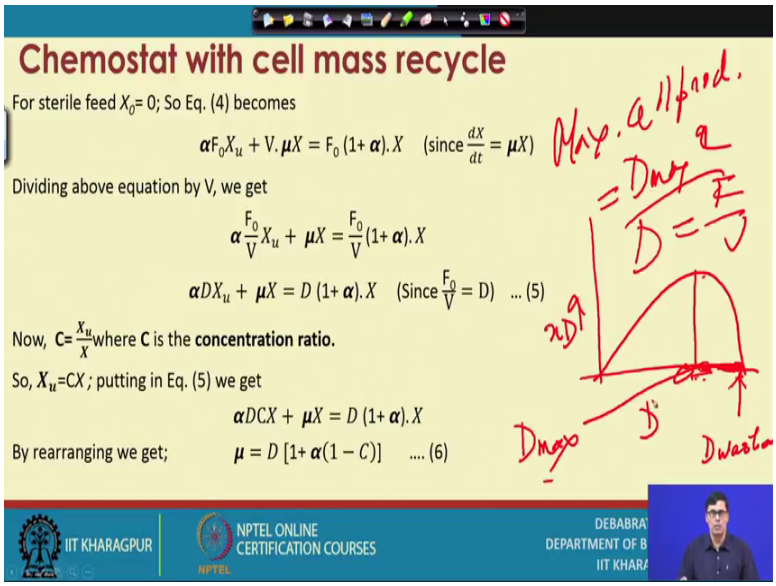
(Refer Slide Time: 13:10)



So, that means, that in a C S T R, under steady state condition and still mu equal to D. And, but here, is mu equal to 0.5; that means, you can if D by mu is there, how much that is 1 by 2.5; that means, this is 4.

That means that, you know I can write mu equal to or I can write D equal to 4 mu. So, I can run this if you do the recycling, I can write 4 times of this; that is, the specific growth of the cells. So, this is the speciality of the recycling of the cells.

(Refer Slide Time: 14:14)

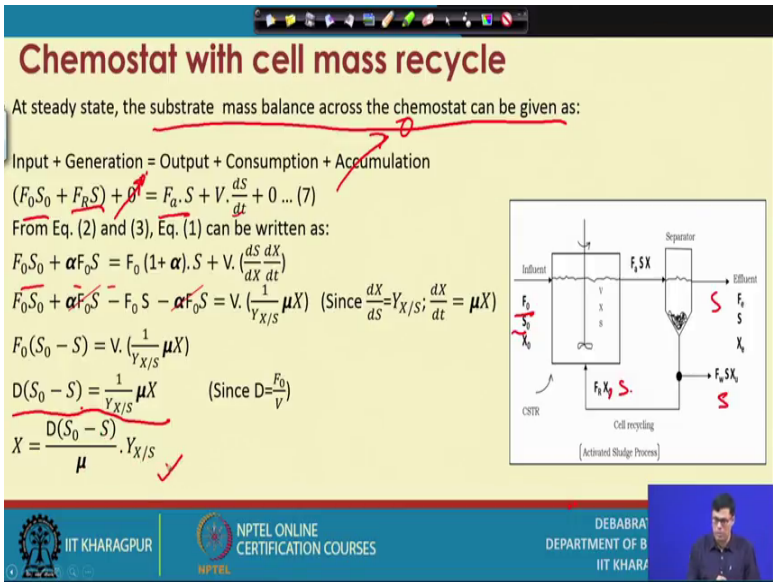


If you recycling of the cells, how you can justify, that we can operate the same system safely now, we have already seen that, when you plot that you know x D versus D, that of what we have done, we have plot like this, am I right? And this is the situation that we have here, this is called D washout.

And at the same time, we find that, here this is what, this is called D max. Now, suppose, in case of bakers, yeast fermentation process, we wanted to have maximum amount of cell mass formation. So, what is the maximum cell productivity? Maximum cell productivity is how much productivity equal to D max into x, where the D max what is the x value that is the maximum. Now, as I told you, that D max and D washout, these 2 they are very close to each other. So, if you increase a little bit the flow rate, because D equal to what F by V.

Now, if you increase the flow rate little bit high, there is every possibility that D (Refer Time: 15:18) max can meet the D washout situation, then we will not get any cell mass in the reactor. Now this system can be safely operated if you recycle the cell. So, even it is the increase little bit, it is not going to effect in your system at all. So, this is how we can safeguard the cell wash out by the cycling of the cells.

(Refer Slide Time: 15:39)

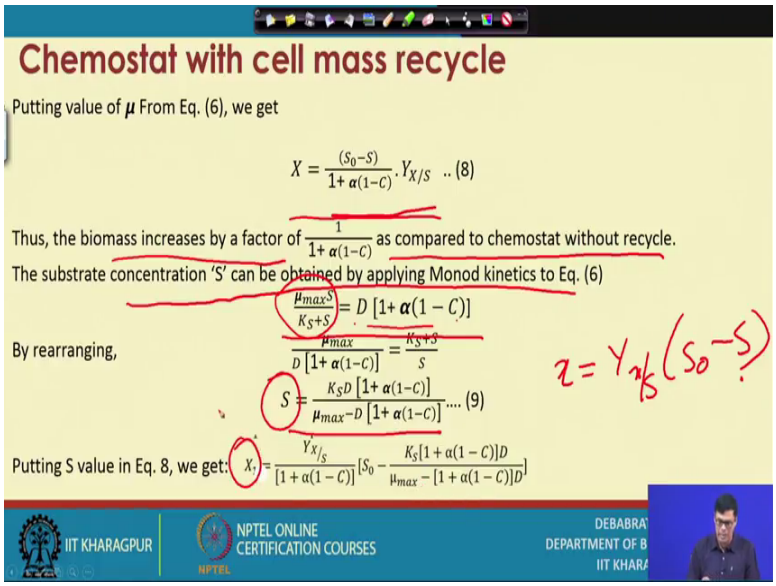


Then we try to find out that, steady state substrate mass balance across the chemostat. We can write F 0. This is F 0 into S 0 F R into S because, we assume here this will be S, so, F R into S here also it will be S.

Here of the everywhere it will be S and this is equal to F into and no generation of the cells that will be substrate. This will be 0 this is F a into S plus that you know rate of substrate that is degraded consumption in the system and rate of accumulation that should be equal to 0.

Now, if you do the analysis of the process like this we will come across these equation dS 0 minus S equal to 1 by X into mu X. So, x will be equal to what dS 0 S mu Y X by S this is cell mass concentration and we can easily determine like this.

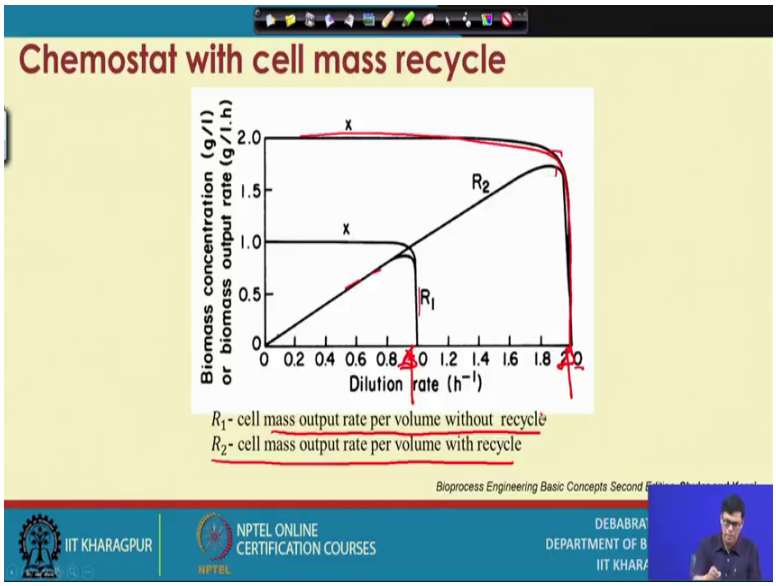
(Refer Slide Time: 16:48)



Now, mu that X equal to this we can see that we wrote like this and before that, we have derived this equation mu equal to D into 1 minus alpha into the 1 minus C. So, this I can put it here and final equation will be this. And then does the biomass increase by a factor of this as compared to the chemostat without recycling. So, this is the comparison that we have made and then substrate concentration can be obtained by applying Monod kinetics like this equation that we have.

And this already, we find this is equal to mu and Monod question is this and then we find out the S value. S value will be this and Y value that the X value will be what Y X by S in 2 S 0 minus S. So, if you put the S value here, then you can calculate the value of X, but both S and X value you can calculate with this, with the recycling system.

(Refer Slide Time: 18:09)

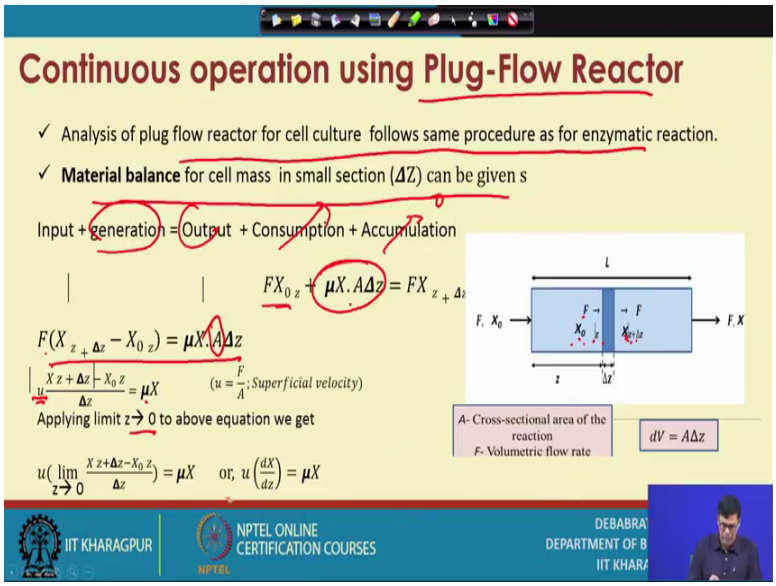


Now, if you plot this dilution rate with the biomass concentration and what will observe what we will get and the rate of biomass formation both say, if you get here, then you will find that, this is the X, this is the true situation.

We have R 1 and R 2. What is the R 1? R 1 is the cell mass output for volume without recycling and R 2 is with recycling. So, with recycling, you would the cell mass with concentration will be like this and you can see that you are D washout. D washout is much higher as compared to without recycling. Without recycling, it may be close to 1 and it is about 2 and R also R 1 that is the cell mass output for without is that has been given here.

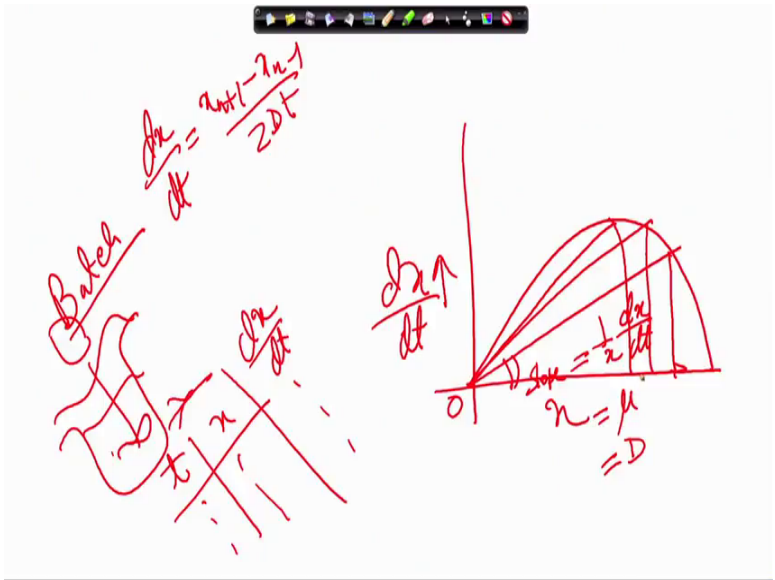
This is the 2 situation that we have rate of cell mass formation. In case of without recycling rate of cell mass formation, in case of with recycling this with recycling, our cell mass growth is increased to a great extent.

(Refer Slide Time: 19:38)



Now, that you know after here, I want to point out one thing; that one thing I point out that you know.

(Refer Slide Time: 19:52)



We have seen the batch process; batch process that is like this.

So, we take the material at a time, allow it to react. After the reaction is over, you take it out. Now, here, with respect to time, you can have the cell mass concentration. So, then we can monitor d X by d t. Though how we can monitor d X by d t? I told you dX by d t equal to X n plus 1 minus X n minus 1 divided by 2 del t. Now if it is like this. Now, if

you plot X dX by d t, mass is X what kind of nature of plot we will get like this. Now, what is the slope? This slope this is 0. So, slope is the tan theta. Tan theta is the altitude by base.

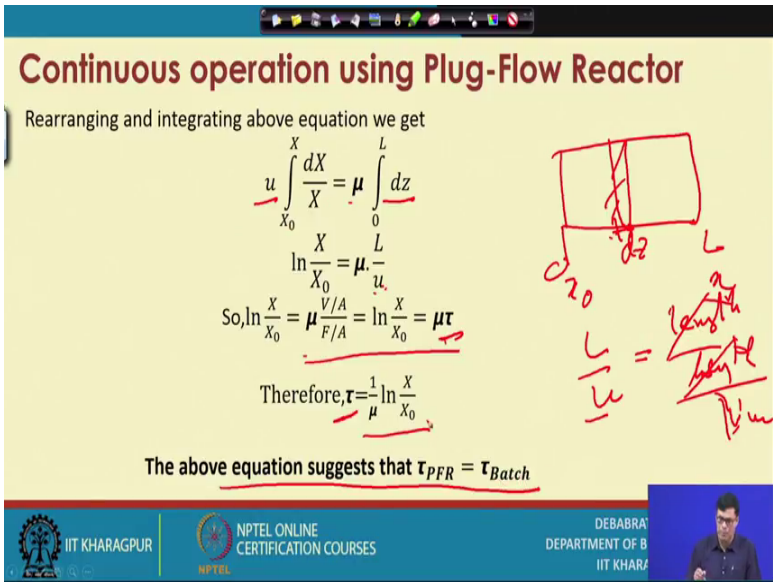
So, it is d X by d t by 1 by X this is equal to slope this in the angle these like this. So, this is nothing but equal to mu and this mu under steady state condition and sterile field in case of V the chemostat this is equal to D. So, the best data that can be possible from the best data it is possible to find out that, what will be the cell mass concentration in a chemostat at a particular dilution rate provided you have the plot of d X versus X. Now if you have this dilution rate, then you can find out the corresponding the cell mass concentration.

Now, if you have this one is dilution rate, you can find the corresponding. So, without going for this operation of the content process, it is possible by simple operation of the batch processes possible to find out what should be the cell mass concentration in a chemostat. Now, let me explain that, how we can analyse the plug flow reactor. It is similar to the plug flow reactor. When we discuss during the in geometric reaction, the analysis of the plug flow reactor plug culture follows the same procedure as the enzymatic reaction. The material balance in this section is like this.

So, I told you this is kind of tubular flow and plug flow is kind of tubular flow. So now, here this equation is like this, that F the F is the flow rate and X is the z here X 0 into z. So, this is z and this is the X z plus X.

So, this is the input and then rate of growth of the cells this is the generation of the cells and then output. And we can assume the consumption. And this, we under steady state condition, this is equal to 0. Then we can write this equation in this form; this is F into X z plus the del z and the X 0 into z and this equal to this is the F divided by A. A is the cross-sectional area. If you divide by this, you will get the velocity. Velocity is that into this equal to u. Now, if z equal to tends to 0, then what we can write, this is equal to we can write that d X by d z u into D that d z equal to mu into X.

(Refer Slide Time: 23:44)

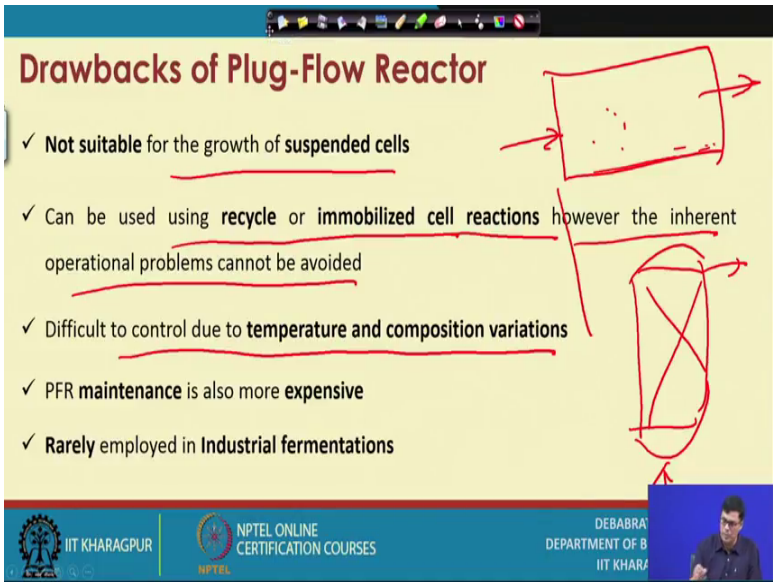


Now, if you do the integration that, you know that u is the velocity and d X by X u will be d z. And so, this is the reactor that we have a strip here. So, this is d z. This is the length is d z.

Now, we integrate it from 0 to 0 to L this is the L. So, if you integrate that, then we will find L by u, you can bring it here and this is X. X 0 they will started with X 0 and finished with X; that is, the cell mass concentration. Then, we can have this is the L by u, then this is this equation and then and L is what L by u. The L what is the unit is it length. And what is the unit of u length per unit time? So, you know length will cancel the time will come.

So, L by u is nothing but tau. So, this we can replace the l n x by X 0 equal to tau. So, we can write the tau that is, the space time of the plug flow reactor is L by mu l n X by X 0. So, this is the same as the expression. If you compare with the batch process, the tau plug flow reactor is equal to tau, the batch process the same expression that we have.

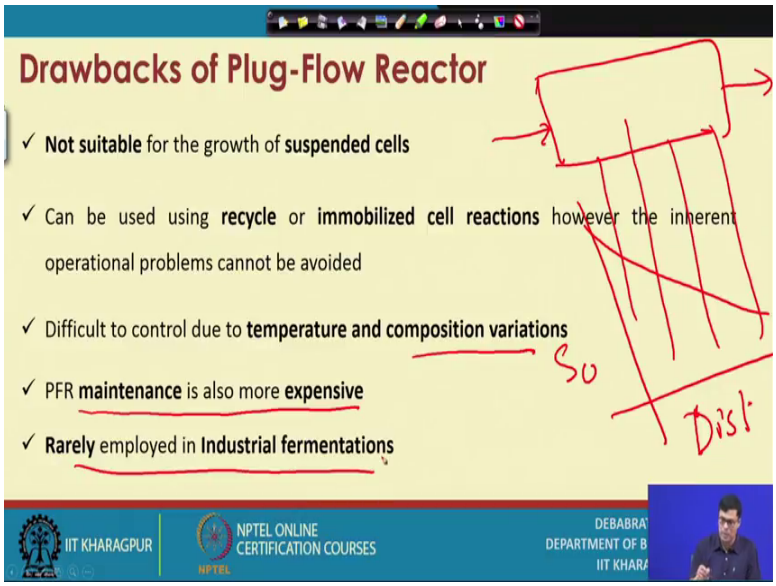
(Refer Slide Time: 25:14)



The drawbacks of the of the block flow reactor, it is not suitable for the growth in suspension because, I told you that in the plug flow reactor, there is no back mixing.

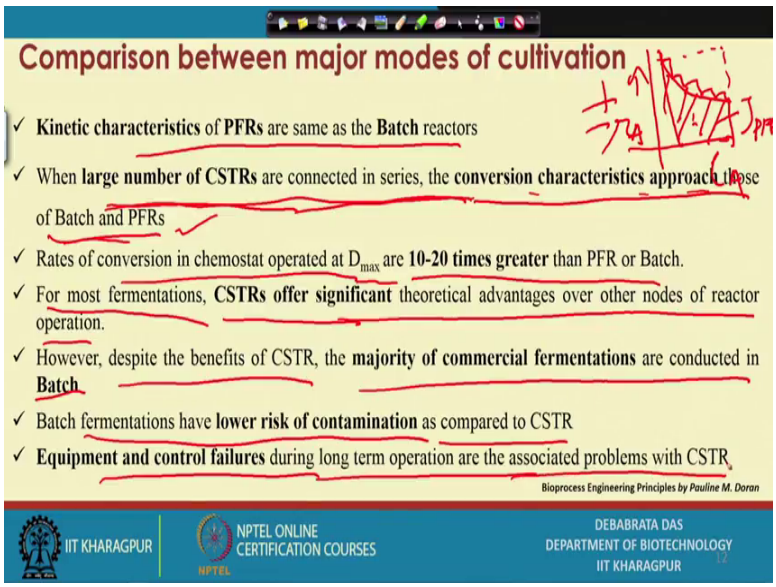
So, if there is a suspended cell, there is every possibility it will settle down. Then, it is not suitable and can be used recycle or immobilized cell reactions. So, suppose, in a column, if you pack the cells in a immobilized column and you pass the substrate here, take out, that is you know, that kind of system we can operate it in the plug flow manner. However, the inherent operational problem cannot be avoided. Because, inherent proper what is the operation problem, there should not be any and that you know back mixing the difficult to control the temperature and composition variation.

(Refer Slide Time: 26:12)



Because, if you suppose, in this reactor, if you make a concentration profile with respect to distance that S 0, then what will be with respect to distance at different distance, your substrate concentration will be different. So, difficult to control the temperature reason is that, there is no hesitation to master it. Heat transfer will not be proper and composition variations of I showed you, how the composition variations is there. Plug flow maintenance is also more expensive and rarely employed for the industrial purposes. So, this is what we have that plug flow reactor is rarely used.

(Refer Slide Time: 26:52)



Now, comparison between the major mode of cultivation, what I lastly pointed out the kinetic characteristics of plug flow reactor is same as batch process. Large number of C S T R connected in series conversion characteristics approaches to. So, those of plug flow reactor I showed you that, when you plot to minus r. R A versus C A in the chemical reaction, I showed you if you have this in case of product change, you know, this is in case of product inhibition. We go for this is tau plug flow reactor. Now, this is can be, but in case of C S T R, it will be area is node.

But, this can be replaced by a multiple C S T R like this. So, you have multiple C S T R, this can be number noise number of C S T R connected in series the conversion characteristic approaches in the batch and plug flow reactor. The rate of conversion of chemostat operation D max in 10 to 20 times greater than plug flow reactor. And batch process for the most fermentation, the C S T R offers significant theoretical advantage over the other nodes of reactor operation with they can be very easily operated as simple stirred tank reactor.

You pass your substrate one end and take out product other end; however, despite the benefit of C S T R, majority of the commercial fermentation is conducted in the batch node because, they always continuous process required some kind of skill of operation. And batch fermentation is lowered risk of contamination as compared to C S T R. This we already pointed out the C S T R.

When you operate for long time, there will be some kind of contamination problem and whereas, in case of batch system, the contamination problem comparatively very less equipment and control failure during the long-term operation are associated with the problem of with the C S T R. So, and this is basically, the instrumental controls. So, if they need any kind of instrumentation failure is there, then the process is going to suffer. So, these are the several problems we face during the operation of C S T R.

So, what I try to point out here that, the major problem that we have with the chemostat process is, the cell mass wasting from the reactor and that can be and meeting the situation like cell washout. And since, that problem can be easily overcome if we recycle the cell in their solid matrix and is recycle the cell in the particular reactor and later on we find that, how we can determine the space time that is required in the plug flow reactor. And finally, we make a clear cut the difference between chemostat at the plug

flow reactor the batch process and what I pointed out that batch process contamination problem will be less. But, you know productivity would be more in the case of C S T R and plug flow reactor will be very difficult to operate.

Thank you very much.