Bioreactor Design and Analysis
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Lecture – 18
Design of Continuous Bioreactors Practice Problems - Part 2

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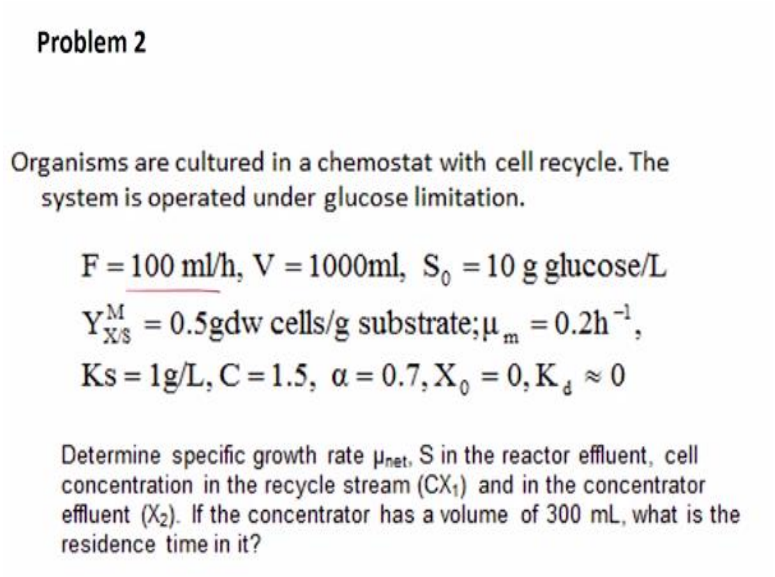

Let us start the problem 2. So, if you can see the problem on the slide, organisms are cultured in a chemostat with cell recycle. The system is operated under glucose limitation. The flow rate is given as 100 ml per hour. The volume of the reactor is 1000 ml. And the feed substrate concentration is 10 grams glucose per liter. The yield coefficient and the Monod saturation constant and maximum specific growth rate of the culture have been provided. And some information regarding the recycle stream is also given.

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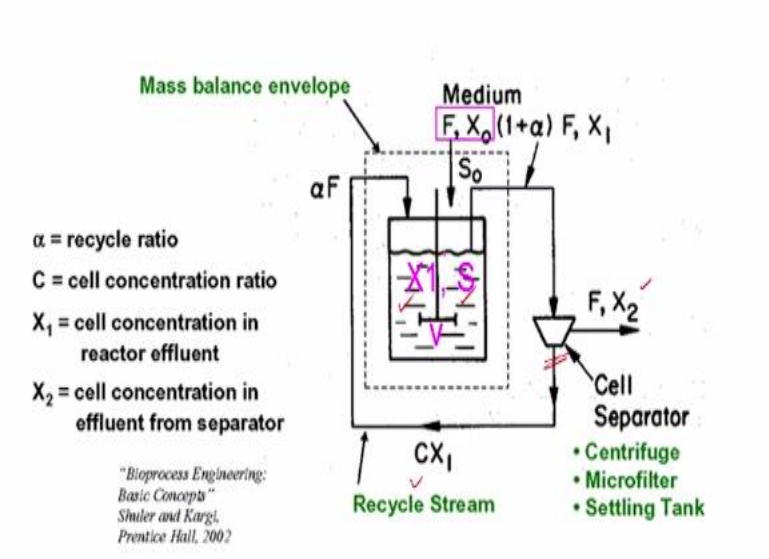

If you see the schematic which has been provided on the slide and the various notations where alpha stands for the recycle ratio, capital C stands for cell concentration ratio in the recycle stream versus the stream which is coming out of the reactor. X 1 is the cell concentration in the reactor effluent which means the stream which is leaving the reactor and X 2 is the cell concentration in the effluent from the separator.

So here this is the cell separating device. So now let us go back to the problem. We need to determine with all these information given the specific growth rate of the culture, the substrate in the reactor effluent at steady state and cell concentration in the recycle stream and also in the stream which is coming out from the cell separating device which means X 2. So, we need to determine X 2, CX 1, S and X 1.

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Solution

Consider material balance on biomass around the fermentor:

$$FX_0 + \alpha FCX_1 - (1+\alpha)FX_1 + V\mu_{net}X_1 = V\frac{dX_1}{dt} \quad (1)$$

Here, α = recycle ratio based on volumetric flow rates
C= concentration factor
F = nutrient flow rate
V = culture volume
Xo and X1 cell concentrations in feed and recycle streams respectively
X2 cell concentration in effluent

At steady state, and sterile feed

$$\mu_{net} = (1+\alpha-\alpha C)D = [1+\alpha(1-C)]D \quad (2)$$

Consider material balance on substrate around the fermentor:

$$FS_0 + \alpha FS - V\frac{\mu_g X_1}{Y^M_{X/S}} - (1+\alpha)FS = V\frac{dS}{dt} \quad (3)$$

So now in order to determine this let us first list down what has been given to us and do a mass balance across this reactor. So, if you see the dotted line it is your system boundary and we will do a mass balance across the system boundary for the biomass. So, if we do the material balance, then FX 0 is the rate at which the biomass is coming inside the reactor. So, if you have noticed in the schematic F is the flow rate of the feed inside the reactor.

And X 0 has been denoted as the feed biomass concentration and S 0 is the feed substrate concentration. So, this is the inlet term. Alpha FC X 1 again is another inlet term which is coming from the recycle stream and this is the rate at which the biomass is going outside the reactor which is the reactor effluent this stream. So, 1 + alpha into F is your flow rate of the exit stream with substrate concentration as S and biomass concentration as X 1.

So, this is the rate at which the biomass is leaving the reactor the third term. And the fourth term is the rate at which the biomass is getting formed inside the reactor. So, the net output is of all these terms will be the rate at which the biomass will be getting accumulated inside the reactor which has been denoted as dX 1 by dt. Now because your X stands for concentration and it is a chemostat, so the volume is constant which has been brought outside.

Now for steady state, the accumulation will become 0 for the biomass. The biomass concentration in the feed can be assumed to be 0. So, if you do the rearrangement and assuming there is no endogenous metabolism present, so mu net is nothing but your mu. So, if we do these equalities and put F by V as notation D in terms of dilution rate with no cell recycle, so then we can determine the correlation in a cell recycle system shown here between the steady state specific growth rate and the dilution rate.

So, at steady state this will be the correlation which will hold true for this system. Now if we do a substrate balance in the same reactor with the same system boundary FS 0 is the rate at which the substrate is coming inside. Alpha FS is the rate at which the substrate will be coming back inside the recycle stream because in the stream the substrate concentration was S and only biomass has been separated, rest of the stream is coming out.

The third term stands for the rate of substrate consumption in growth. The fourth term is the rate at which the substrate is going outside in the reactor effluent in the stream and the net of the right hand side terms will be what will be getting accumulated inside the reactor. So, dS

by dt stands for the rate at which the substrate is getting accumulated inside this reactor with cell recycle stream.

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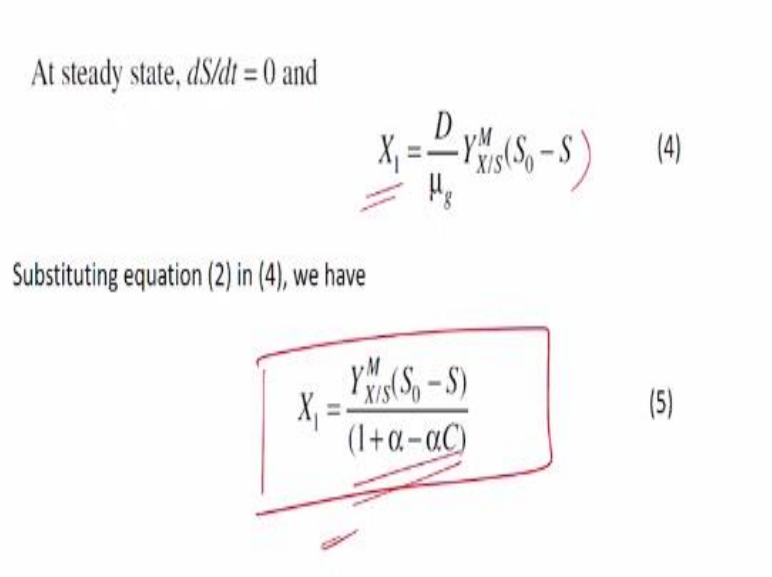

So, at steady state again no accumulation and if you do the rearrangement putting F by V as D, then the correlation at steady state says that the biomass will be a function of alpha and C as well unlike the chemostat in the absence of any recycle stream.

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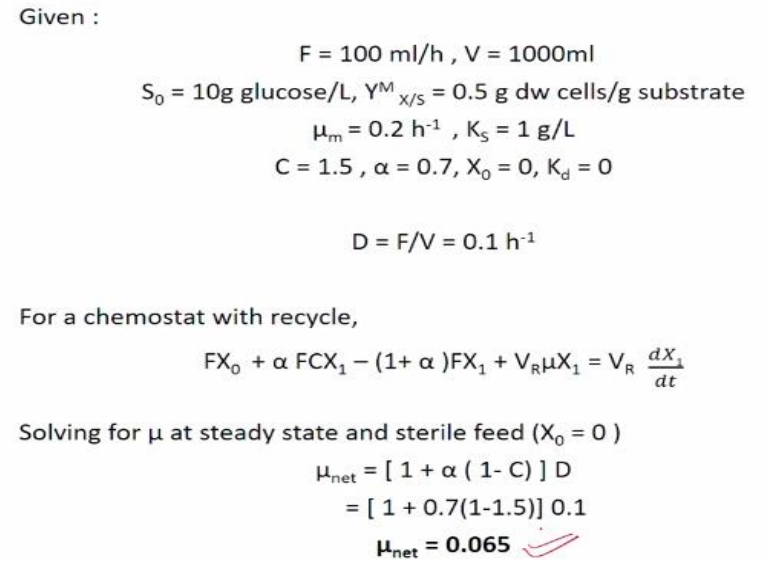

First we need to find the net specific growth rate at the steady state which here it has been determined as 0.065.

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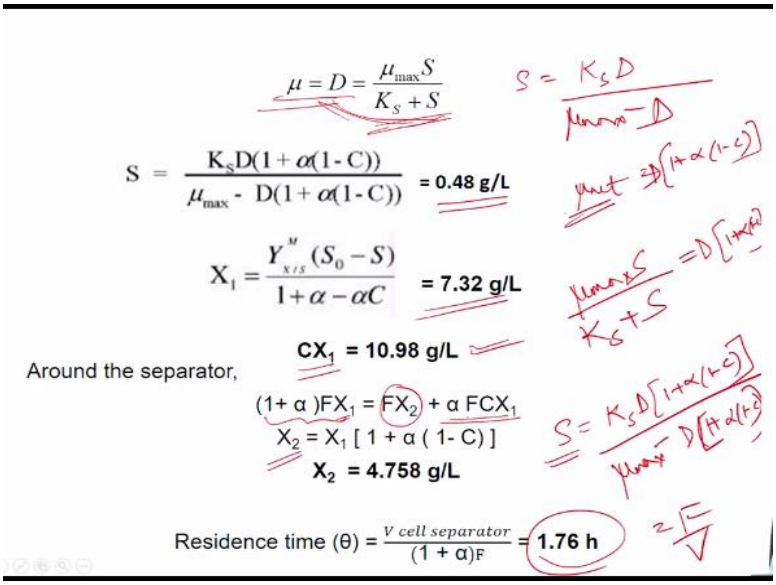

Then assuming that the specific growth rate of the culture is following Monod's model. So if you remember for a chemostat without cell recycle mu was equal to mu max S upon K s + S which is equal to D in case of steady state. And then finally the rearrangement would lead to an expression to determine S in terms of the dilution rate which used to be K s D upon mu max – D.

Now however, in this case if you do the rearrangement so in this case it is mu net equals to the expression is here 1 + alpha times 1 – C into D. Now this mu net we know is the specific growth rate of the culture which is following Monod's model with no endogenous metabolism. Now if we do the rearrangement the expression for S can be obtained similarly as K s D divided by mu max – D.

So now we know all the terms here and the dilution rate D = F by V. So we need to know the F, F is given in the problem as 100 milliliter per hour. So, if we know all the terms and the volume is also known, so we can find the dilution rate which has been calculated here as 0.1 hour inverse. Now once we know the dilution rate, we have also calculated mu net and then we can calculate the substrate concentration as well.

So now the substrate concentration calculated using these terms comes out to be 0.48 grams per liter. Now using the residual substrate concentration if you remember by substrate balance, we determined at steady state the expression for the correlating biomass with the substrate concentrations. So, using that correlation we can determine the value of steady state biomass concentration and in the recycle stream it is CX 1.

So now once we know the X 1 the biomass present inside the reactor at steady state, we can find the concentration of biomass in the recycle stream. Now let us do a mass balance across the cell separator for biomass. So, if this is the cell separating device the mass balance across the separator would reveal 1 + alpha times FX 1 this is nothing but the rate at which the biomass is coming in the separator.

FX 2 is the rate at which the biomass is leaving the separator for harvest and alpha F into CX 1 is the rate of the biomass at which it is coming back inside the reactor as a recycle stream. So, with this equality in place, we have now determined X 1, we know F, we know C, we know alpha, we can determine now the value of X 2 which is the biomass concentration leaving the cell separating device.

Now residence time in the cell separator would be V by F. So the volume in the cell separating device divided by the flow rate, flow rate incoming and outgoing will be the same which is 1 + alpha times F and the volume of the cell separator was given in the problem which is 300 ml. So, we can determine the residence time V by F, F is the flow rate in the cell separating device, so which comes out to be 1.76 hours.

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Problem 3

Suppose you have a microorganism that obeys the Monod equation:

$$\frac{dX}{dt} = \frac{\mu_{max} * S * X}{K_s + S}$$

where μ_{max}= 0.7 $h^{-1}$and K_S = 5 g/L. The cell yield ($Y_{X/S}$) is 0.65. You want

to cultivate this microorganism in either one fermenter or two in series. The flow rate and the substrate concentration of the inlet stream should be 500 L/h and 85 g/L, respectively. The substrate concentration of the outlet stream must be 5 g/L.

a. If you use one CSTR, what should be the size of the fermenter? What is the cell concentration of the outlet stream?

b. If you use two CSTRs in series, what sizes of the two fermenters will be most productive? What are the concentration of cells and substrate in the outlet stream of the first fermenter?

c. What is the best combination of fermenter types and volumes if you use two fermenters in series?

Let us see the third problem. Suppose you have a microorganism that obeys the Monod's equation, so I hope you can understand from where this dX by dt is coming. So in turn this is 1 by X, so this X goes here. So, this is nothing but 1 by X dX by dt which has been rearranged where mu max and K s values have been provided in this model. The cell yield is

0.65. Now one would like to cultivate this microorganism in either one fermenter or 2 fermenters in series.

The flow rate and the substrate concentration of the inlet stream should be 500 liters per hour and 85 grams per liter respectively. So, the substrate concentration of the outlet stream is also defined which should be 5 grams per liter. So, the conversion has been defined. If you use one CSTR, what should be the size of the fermenter? What is the cell concentration of the outlet stream? Okay.

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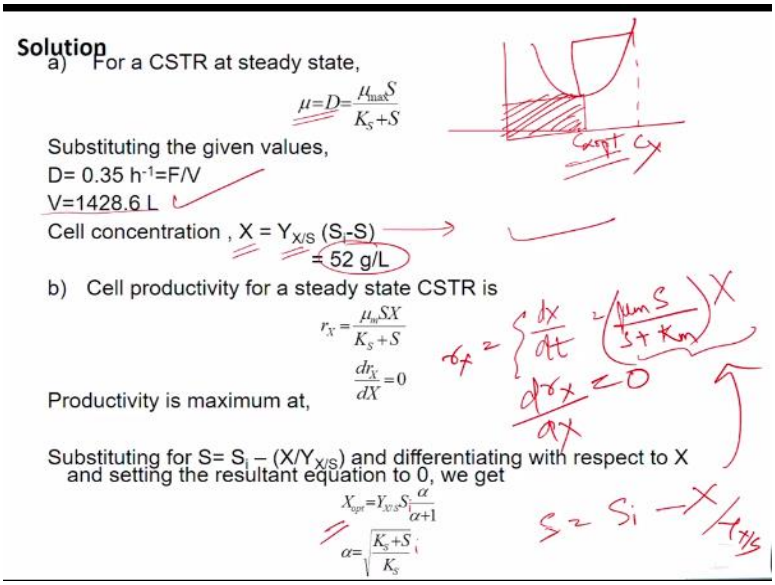

So now let us do the first part. So, if you are using a CSTR and there is a single CSTR being used, so then at steady state specific growth rate is equals to dilution rate. The culture is following Monod's model. Let us see what is given to us. Flow rate has been given. So now once the flow rate is given and we have been given the value of S inside the reactor coming outside the reactor which is 5 grams per liter.

So, which means steady state substrate concentration is 5 grams per liter. So, your biomass which can be obtained, the X can be obtained using the substrate balance at steady state given the value of Y x by s, i is also given, s is given, Y x by s is given. So, we can find out the steady state biomass concentration. Now mu max and K s was also given, so we can determine the value of mu using the steady state substrate concentration, which will be equal to dilution rate at steady state.

Once we know the dilution rate, we have the value of F, so we can determine the volume of the reactor. Now what is the cell concentration of the outlet stream? So, we have now determined both the parts, the size of the reactor and the biomass at steady state. Let us see the second part. If you use two CSTRs in series what size of the two fermenters will be most productive? What are the concentrations of cells and substrate in the outlet stream of the first fermenter?

So now given is that we are using two CSTRs in series and the value of the substrate coming outside the second reactor will be 5 grams per liter and the flow rate in each of these reactor inlet and outlet they being CSTRs is 500 liter per hour. The condition is that the two fermenters should be most productive. So, which means we need to find the size of the two reactors for maximum biomass productivity.

What is the concentration of cell in the outlet stream of the first fermenter is also to be determined? So, let us first try to find out what should be the dilution rate at which maximum productivity can be obtained. So, it is being operated for maximum productivity. So, we know r x is nothing but dX by dt which is mu m S upon S + K s by Monod's model. This is mu times X.

Now we know to find maximum productivity this r x, if this is r x it has to be again differentiated with respect to X and should be put to 0 to determine that value of x at which r x will be dr x by dx would be 0. So, this is to find the maximum r x. Now in this expression, we see there are two variables S and X. So, this differentiation is only possible if we bring this expression in only in terms of one variable.

So, we use another correlation using the yield coefficient to substitute S in terms of X. So if we substitute this here, then we differentiate it and find the value of X optimum, the biomass productivity or the biomass concentration for maximum productivity. So, your x optimum comes out to be Y x by s times S the residual substrate concentration alpha upon alpha plus 1. So, the residual substrate concentration is known and the value of K s is also known. So, we will now find out the value of alpha.

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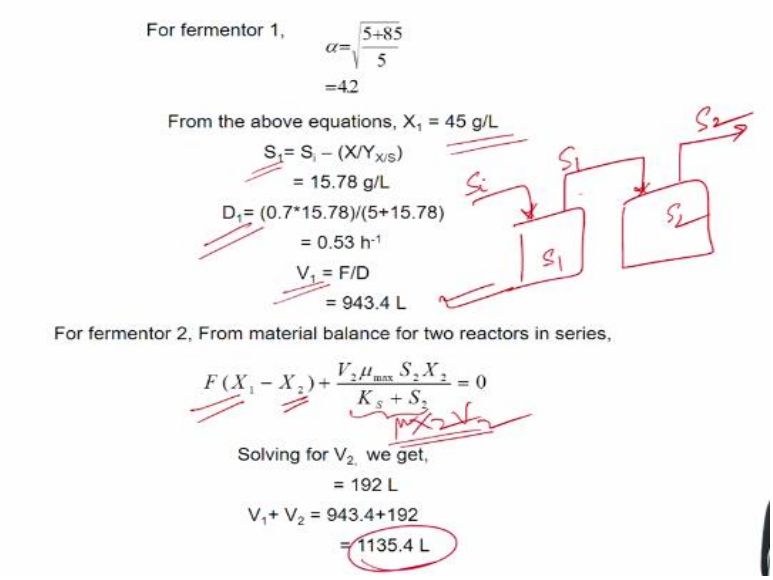

Once alpha is known, we can find the value of X for the maximum productivity. We determine the value of residual substrate concentration using the biomass. So now if you notice this S 1 is the stream which is coming out from the first reactor and if two reactors are in series. So, we have come to know the optimum biomass for the reactor in the first reactor.

So now we can find out the biomass from the first reactor and the substrate which is coming out from the first reactor. Once we know the substrate, we can find out the dilution rate because it is equal to specific growth rate. So, if we know the substrate concentration inside this reactor, we can find out the specific growth rate which will be equal to the dilution rate given as D 1. Once we know the dilution rate, we can find the volume of the reactor given the flow rate.

Now for the second fermenter if you do the material balance for the two reactors, this term is Monod's model. This is the rate at which for the biomass is getting formed. This is the rate of input and this is the rate of output F X 2. At steady state no accumulation. So, from this balance we know rest of the terms we can calculate the volume V 2.

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c) For the best combination of fermentors, it's a CSTR followed by Plug Flow Reactor (PFR)

So, V_1 is same as calculated above,

For PFR connected to a CSTR, τ_2 can be calculated as

$$\tau_2 \mu_{max} = \left(\frac{K_S Y_{X/S}}{X_1 + S_1 Y_{X/S}} + 1 \right) \ln \frac{X_2}{X_1} + \frac{K_S Y_{X/S}}{X_1 + S_1 Y_{X/S}} \ln \frac{S_1}{S_2}$$

$= 0.32h$

$V_2 = F/D_2$

$= 160$ L

Total volume = $V_1 + V_2 = 943.4 + 160$

$= 1103.4$ L

Third part what is the best combination of fermenter types and volumes if you use two fermenters in series? Now for the best combination it is a CSTR which should be followed by a PFR that we know. Now, so V 1 will be the same as we calculated in the first part of the reactor, first reactor in the second part while for the second reactor because it will now follow batch kinetics the PFR.

The PFR design equation is shown here where if we know the final biomass in the two reactors, the substrates in the two reactors and the yield coefficients are known, then we can determine the value of tau 2 which is the residence time in the second reactor. Once we know the resonance time in the second reactor which is F by V is equal to dilution rate we know this, now residence time is V by F.

Once we know the residence time, we can calculate the value of V 2 as we know F. So, total volume will be the volume of the CSTR as we had determined here plus the volume of the second reactor. So, you can see that it has reduced from the combination of two CSTRs. Now we know that for the combination of two CSTRs to achieve maximum productivity, the first should be run at C X optimum and then the second should be run till the desired C X.

So, we need to find for C X optimum which will become the inlet for the second reactor because this will be the most productive fermenter with minimum residence time. So, the productivity would be maximum.