Material and Energy Balances
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Module No # 06
Lecture No # 29
Material Balance: A Review – Part 3

Hello everybody, welcome to today's lecture on continuing the review for material balances, we had performed material balance calculation and we reviewed the concepts using one example for chemical reactions. Today we will review the fundamentals associated with the bio chemical reactions using one example problem which covers all the aspects of bio chemical reactions with respect to cell cultures and we will also talk about recycle as a part of this problem.

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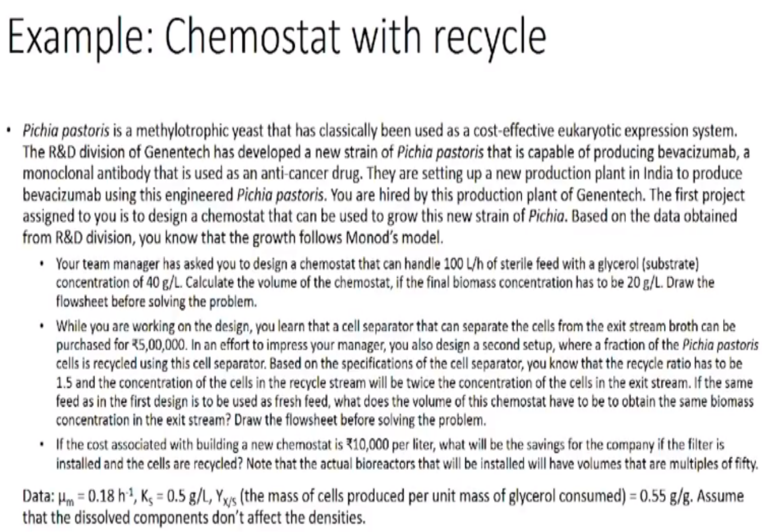

Let us go to this example problem Pichia Pastoris is a methylotropic yeast that has classically been used as a cost effective eukaryotic expression system. The R & D division of Genentech as developed a new strain of new Pichia Pastoris that as a capability of producing Bevacizumab a monoclonal antibody that is used as an anti-cancer drug. They are setting up a new production plant in India to produce this drug using the engineered Pichia Pastoris.

You are hired by this production plant of Genentech the first project assigned to you is to design a chemostat that can be used to grow this new strain of Pichia. Based on the data obtained from the R and D division you know that the growth follows Monod's Model. Your team manager has asked you to design a chemostat that can handle 100 liters per hour of sterile feed with glycerol as the substrate with the concentration of 40 grams per liter.

Calculate the volume of the chemostat if the final biomass concentration has to be 20 grams per liter draw the flow sheet before solving the problem. While you are working on the design you learnt that a cell separator can separate the cells from the exit stream broth can be purchased for 5 lakh rupees. In an effort to impress your manager you also design a second setup where a fraction of Pichia Pastoris is recycled using this cell separator.

Based on the specifications of the cell separator you know that the recycle ratio as to be 1.5 and the concentration of the cells in the recycle stream will be twice the concentration of the cells in the exit stream. If the same feed as in the first design is to be used as fresh feed what does the volume of this chemostat have to be obtained the same bio mass concentration in the exit stream. Again draw the flow sheet before the solving the problem.

If the cost associated with building a new chemostat is rupees 10000 per liter what will be saving for the company if filter is installed and the cells are recycled. Note that the actual bio reactor that will be installed will have volumes that are multiples of 50 data given is Mu M is 18 hour inverse KS is 0.5 grams per liter and YXS is 0.55 grams per gram. So you can assume that the dissolved component do not affect the densities.

Now that all the information is available to you in the problem let us first start with drawing the flow sheet. For the first system it is quite simple you have a single standalone chemostat with the sterile feed and one exit which would contain the bio mass an dun utilized substrate. However for the second set up you would have a reactor and cell separator with a recycle stream.

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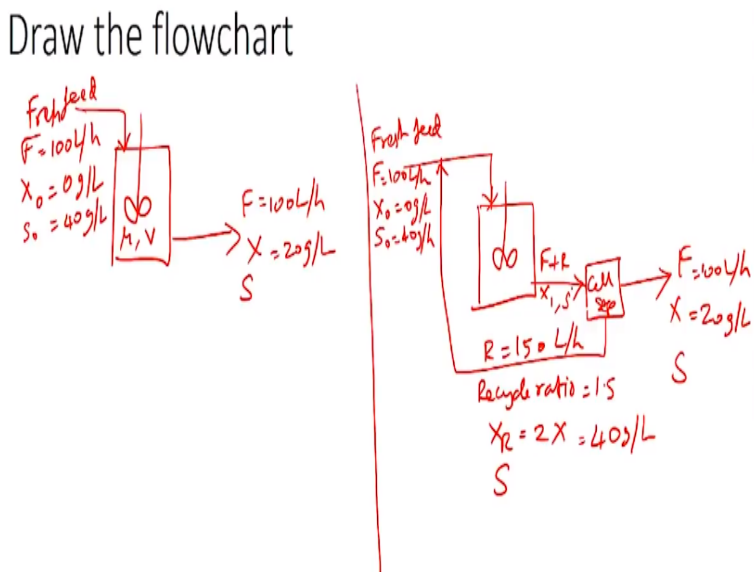

Let us see how these flowchart would look and you will try to label all the streams so that we can perform the calculation. For the first set up you have the chemostat so this is the representation for a chemostat as 1 input and 1 output the input is fresh feed which is coming in at a rate of F = 100 liters per hour we also been told that it is sterile field which means X naught = 0 grams and subset concentration in this stream has been given as 40 grams per liter the substrate used here would be glycerol.

So let us assume that the cells are growing inside the reactor with the specific growth rate of Mu which follows Monod's Model and the volume of reactor would be B. So you have an exit stream for steady state condition the exit flow rate it would also be F which would equal to 100 liters per hour and we have been told that the desirable bio mass concentration would be 20 grams per liter.

We also know there will be unutilized substrate which is glycerol leaving the system however we do not know the concentration of this substrate. Now that we have drawn the flow sheet for the first setup let us try to draw the flow sheet for the second setup. For the second setup you have the chemostat in addition to this you also have a cell separator from which the recycle is sent back to the fresh feed.

So we have a fresh feed so we have been told that this fresh feed has the same properties as the feed of the previous set up. So we know that F here would be 100 liters per hour and this is a sterile fresh feed so X naught = 0 grams per liter and substrate concentration would be 40 grams per liter. You have an exit stream which is taken to the cell separator and the desired products are taken out you also have a recycle stream which is sent back to the mixed with the fresh feed to form the total feed.

So this recycle stream we have some information about this we know that recycle ratio = 1.5 so as we know that the fresh feed is coming at volumetric flow rate of 100 liters per hour we can assume that the recycle would be 1.5 times F which is based on the recycle ratio. So this recycle stream is flowing in at the rate of 10 liters per hour we have also been told that the bio mass concentration in the recycle stream is twice that of the bio mass concentration in the final exit stream.

So this would be 2X and this would also have some unreacted substrate accompanying the recycle stream so your final exit stream we know that would have to have a flow rate of F which equals 100 liters per hour so that you can maintain steady state the bio mass concentration has been given as 20 grams per liter and this would also have unreacted substrate which is at a concentration of S which we need to calculate.

Because we now know the value for X we can calculate the bio mass concentration in the recycle stream as 40 grams per liter. So this stream which leaves the chemostat and enters into the cell separator would have a flow rate of F + R and it would have bio mass concentration of X1 and is dissolve substrate concentration of S. Now that we have all the information regarding both the system let us try to solve this problem using the fundamental we have learnt for bio chemical process.

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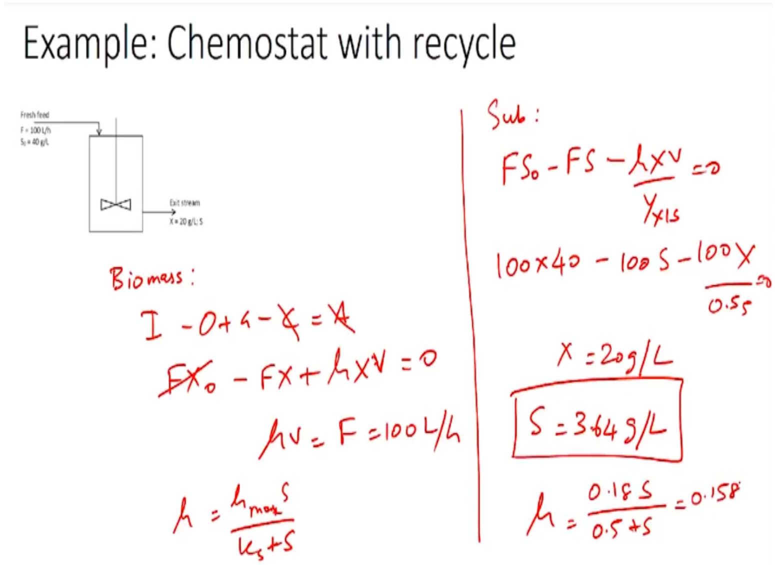

For the setup one let us write a biomass balance input – output + generation – consumption = accumulation at steady state we do not have any accumulation we also do not have any a consumption consuming that there is no cell death. So we are only left with generation input and output.

So input term would be FX naught which goes to 0 because X naught is 0 in the sterile feed you have FX as your output and generation if you remember is given as Mu X V where Mu is the specific growth rate X is biomass concentration in exit stream thereby the biomass concentration inside the reactor also V is the volume of the reactor this equals U. So from this we know that Mu Z V = F which equals 100 liters per hour.

So now this can actually be substituted to your Mu max X divided by KS + S which is the molar smaller if we know the value for yes we can calculate Mu once we have the value for Mu we will be able calculate the volume of the reactor for this we need to write a substrate balance would be FS naught – FS – Mu X V divided by YXS = 0. So FS naught is the substrate coming in FS is the substrate leaving an d- Mu XV divided by YXS is the substrate which is being consumed there is not generation or accumulation term for the substrate.

So substituting the values for FS naught and Mu V and also for X and YX we can calculate the value for S. Let us do that so FS 100 times S naught which is 40 – 100 times S – Mu times V which is calculated as 100 which is F and this times X divided by Y axis which is 0.55 = 0. We know that the desired S is 20 grams per liter substituting here to this equation we can calculate S as 3.64 grams per liter.

So now that we have substrate concentration we can calculate the value for Mu by substituting the value of Mu max which is 0.18 times S divided by KS which is 0.5 + S. This gives you the value of 0.158.

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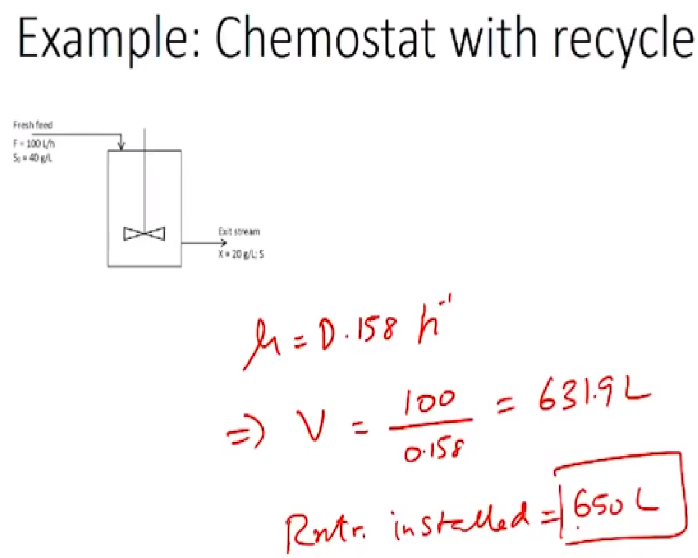

So now that we have the value for Mu as 0.158 hour inverse we can calculate the value for the volume as 100 / 0.158 which is basically F divided by Mu giving you a value of 631.9 liters. As we have been shown that told that the chemostats are available only in the multiple of 50 so the reactor which has to be installed would be 650 liter reactor. So this is the volume of the reactor which is required if you do not have any recycle.

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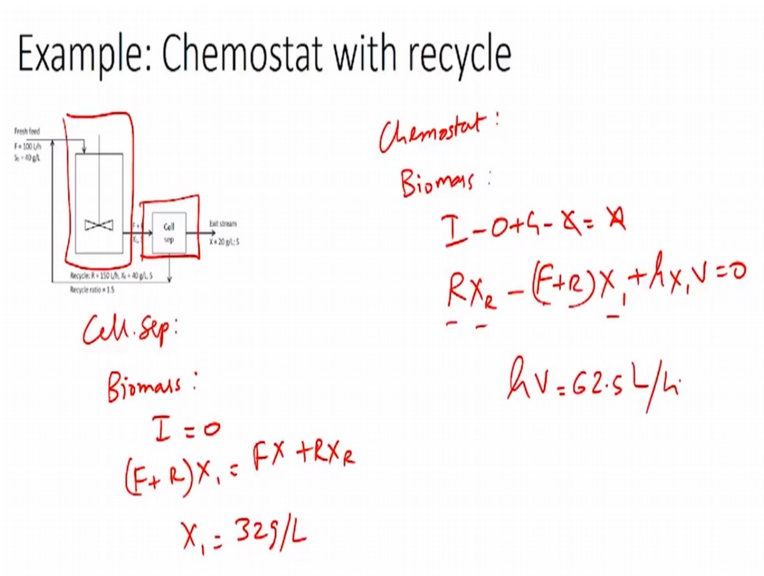

Let us now consider the value the second part of the problem where we have to account for the recycle stream also so in this system we have toe chemostat and the cell separator so for performing the balance equations for the chemostate we need to know the biomass which is leaving chemostat which would be the biomass concentration inside the chemostat also.

This information is required for us to calculate how much bio mass is present how much substrate is present would actually be leaving the system there by calculate the specific growth rate inside the chemostat. For getting the information about the bio mass concentration in this steam leaving the chemstat we have to choose self-separator as the system for writing the balance equations.

Let us take the self-separator as the system of interest for the cell separator let us write a balance equation for bio mass. So the bio mass balance for self-separator would be input = output so input is F + R times X1 and your output would be F times X + R times XR. We know that the value of X is 20 grams per liter and value is SR is 40 grams per liter we also know the values of F and R.

So substituting all of them here we can calculate X1 as 32 grams per liter now that we have the information about the total product stream which is leaving the chemostat we can state writing the balances for the chemostat itself. So for the chemostat we can write a bio mass so the bio mass balance for the chemostat would be input – output + generation – consumption = accumulation.

No accumulation and consumption you assume to that there is not cell death so consumption goes to 0 so input is you do not have any input through the fresh feed you have only input through the bio mass stream. So you have R times XR as the biomass entering through the gross feed into the chemostat. Your gross product steam contains F + R times X 1 biomass generation of the bio mass in the chemostat would be Mu times X1 times V which equals 0.

So now that we have this equation we already know the values for R XR F and X1 so we can calculate the value for Mu V. So from this equation we get Mu V = 62.5 liters per hour.

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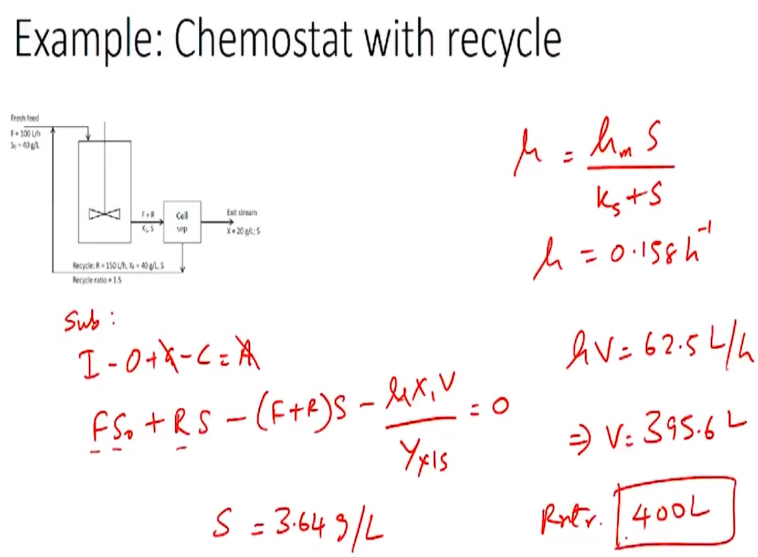

So now we have written the balances for bio mass so next step is to write the substrate balance. So for the substrate balance again there is input – output + generation -consumption = accumulation. No consumption are generation for the substrate so you are left with only input output and consumption input for substrate would come through both the fresh feed and through the recycle feed.

And you have FS naught though the fresh feed + R times S in our recycle feed and you have exit stream as F + R times S and you would have consumption given in terms of Mu X1 V divided by YXS. So again we are assuming that there is no other consumption or substrate other the to form the biomass this equals 0. So we already know the value for F S naught R and Mu times V X1 and Y axis.

Having all these value we can calculate the value for S as 3.64 grams per liter now that we have the value for X we can substitute it back to the Monod's Model Mu = Mu max X divided by KS + S. So knowing the value for Mu max KS we can calculate Mu as 0.158 hour inverse. So we know Mu ZV = 62.5 liter per hour so this implies V would be 395.6 liters. So the reactor which would be used which have to be purchased to be 400 liters

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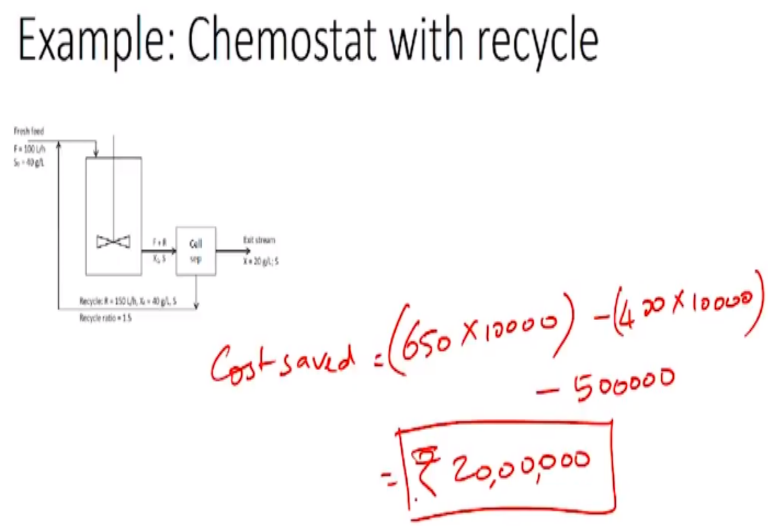

For the first setup we had calculated the reactor volume to be 650 liters and for second set with recycle we have calculated the reactive volume to be 400 liters from these two values we know that there is significant cost saving so we need account for how much cost can be saved by using this recycle stream. So cost saved would basically be 650 times 10000 which is the cost for per liter reactor of a chemostat – 400 times 10000.

So this is the difference for the cost for the reactor in the cost saved we should deduct the cost associated with the purchase of cell separator so this would give us the total cost saved of 20 lakhs. So by using this cell separator and a recycle stream we would be able to save 10 lakh rupees just for the installation process. Obviously there will be more savings due to the operational cost being cut down as the cells are being recycled.

With this we come to the conclusion of the review for the basic aspect of material balances thank you and good bye.