**Bioreactors**
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**Lecture – 12**
**Bioreactor analysis: chemostat and fed-batch**

Welcome to lecture 12 NPTEL online certification course on bioreactors. In the last lecture, we had solved one of the practice problems.

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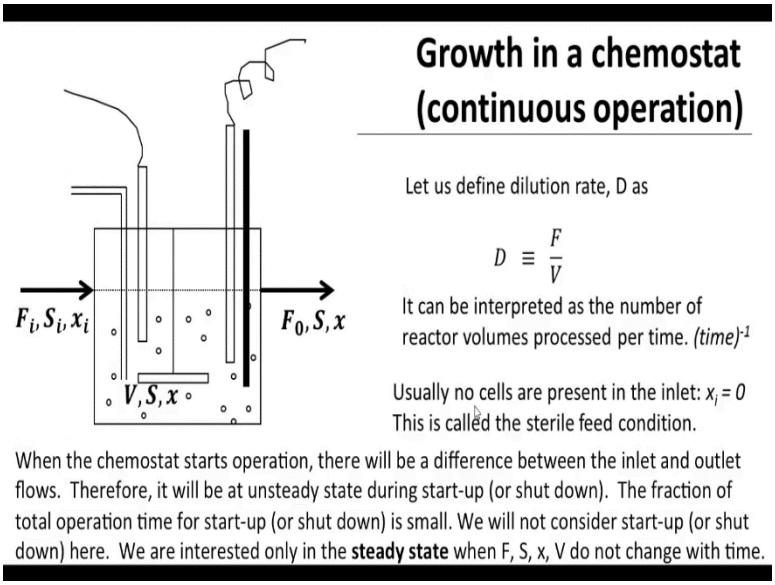


In this lecture number 12, let us continue the module 3 which is on analysis of the common bioreactor operation modes. In the earlier lecture, we saw the batch mode, in this lecture we will see the continuous mode and as mentioned earlier, the continuous stirred tank bioreactor is also called the chemostat- very common term used for the continuous stirred tank bioreactor. So, we will look at growth in a chemostat. The representation of a continuous stirred tank bioreactor or a chemostat is given above, you have the stirred tank, with the stirrer, the vessel, the broth or the liquid in which the cells are growing. The cells are actually magnified in this figure; you won't be able to see the cell as individual cells normally.

We have various measurement probes probably the DO, the pH and the temperature probes and we have aeration assuming that this is an aerobic bioreactor. Then there is a continuous stream of input and a continuous stream of output and let us say that the feed rate, the volumetric feed rate volume per time at the inlet is F i, the substrate concentration in the feed is S i and the cell concentration in the feed is x i. The same parameters in the outlet of the bioreactor F naught or F o. F o is the outlet feed rate volumetric feed rate volume per time, the S is the substrate concentration here and x is the cell concentration here and the volume of the broth, which we will take as the system is V, as you can see a part of this is being pulled out. So, the substrate concentration and the cell concentration here need to be the same as in the outlet stream because what is being inside well mixed is being pulled out. Therefore, the concentrations of these two would be the same.

Now, let us define something called a dilution rate (D) as the volumetric feed rate (F) divided by the volume of the broth (V).

$$D = \frac{F}{V}$$

So, can you make sense of this certain feed rate divided by the volume? So, if you visualize it as certain feed rate divided by the volume, the dilution rate can be interpreted as the number of reactor volumes processed per unit time. So, it needs to have a unit of time inverse. Just think about it a little bit, see whether you are comfortable with the interpretation of number of reactor volumes per processed per time. Usually, there are no cells that are present in the inlet of the chemostat and this condition is actually called a sterile feed condition. In the feed there are no cells, it is sterile and therefore, it is a sterile feed condition. This is the usual mode of operation unless you are using a chemostat in a different way and so on, maybe attached to another chemostat or attached to another bioreactor. Those are different. Stand alone chemostats usually do not have an inlet cell concentration; they are just substrate in the inlet stream.

Now, I would like you to understand this a little better, usually there is a lot of confusion. When the chemostats starts operation, we will have a vessel here you are starting in a

feed here, there is nothing before that and so on. There will certainly be a difference between the inlet and outlet flows, we are not denying that. Therefore, till the flows can be adjusted which could probably take a few hours to be the same and for the conditions to settle down to the steady state conditions, there will be variations in the inlet and outlet. We are not looking at that period at all, those are the start up conditions where there will be unsteady state and there will be shutdown conditions. When you shutdown the bioreactor, it needs to be done in an appropriate fashion and then the process will not be a steady state. If you leave out these two which probably will be a short duration compared to the overall duration of the chemostat, most of the duration it is going to be at steady state and we are going to look only at the steady state part of the operation of the chemostat here, you need to keep this in mind. So, this is what is said here, the fraction of total operation time for start up or shut down is small. We will not consider the start up or shut down here, we are interested only in the steady state. When F, S, F is the volumetric feed rate, S is the substrate concentration at the outlet; x is the cell concentration in the outlet and the volume of the broth, all these variables do not change with time. This is the condition that we are going to analyze and which is the condition under which the continuous bioreactor or the chemostat will be in operation for most of its useful period.

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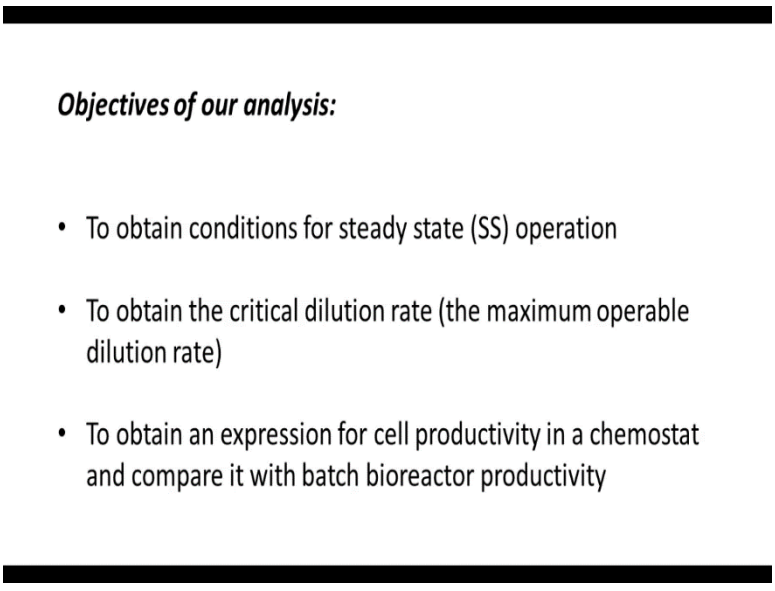

The objectives of our analysis would be as follows: To obtain the conditions for steady state operation, to obtain the critical dilution rate the maximum operable dilution rate, to obtain an expression for cell productivity in a chemostat and compare it with the batch bioreactor productivity might be in for good amount of surprise here.

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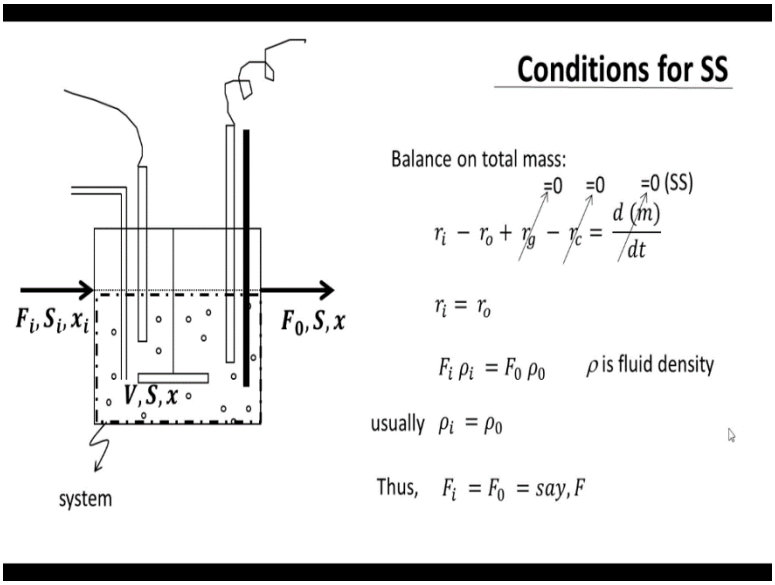


Let us begin the analysis. This is the same chemostat as was shown earlier, the inlet at Fi Si xi, usually xi is zero and the outlet at $F_0$ the volumetric flow rate, S and x are the substrate and cell concentration respectively. Here the volume of the broth is V, which is also the system we have chosen indicated by these dotted and dashed lines and S and x are the same as in the outlet system as shown. Let us do a balance on the total mass the huge tools that we have is mass balance. So, this is the mass balance, by now we should be familiar with rate of input minus rate of output plus the rate of generation minus rate of consumption equals the rate of accumulation of mass we are doing it on total mass.

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

Since we are doing it on total mass, there can be no generation, there can be no consumption, total mass is always conserved, therefore only input and output. We said

that we are interested only in this steady state condition, thus any time derivative can be blindly set to zero. So, this term goes to zero by the definition of a steady state. Therefore, rate of input must equal the rate of output, this is total mass.

$r_i = r_o$

What is rate of input in terms of our volumetric flow rate? A volumetric flow rate is volume per time. We are looking at mass per time here therefore; if we multiply the volumetric flow rate by the density we would get our mass rate.

Therefore, $F_i \rho_i = F_0 \rho_0$ , $\rho$ is fluid density

$\rho_i$ is the density of the incoming stream fluid, $\rho_0$ is the density of the outgoing stream.. Usually, there is no difference in densities between these two streams. We are operating at room temperature and at standard conditions may be 30 degrees, 37 degrees c, one atmosphere pressure it is not easy to get a difference in densities between these two streams under those conditions and given the contents of the system. Therefore, $\rho_i$ and $\rho_o$ can be canceled out.

$F_i \rho_i = F_0 \rho_0$

$F_i = F_0 = F$

So, through a mass balance we got that the flow rates need to be equal.

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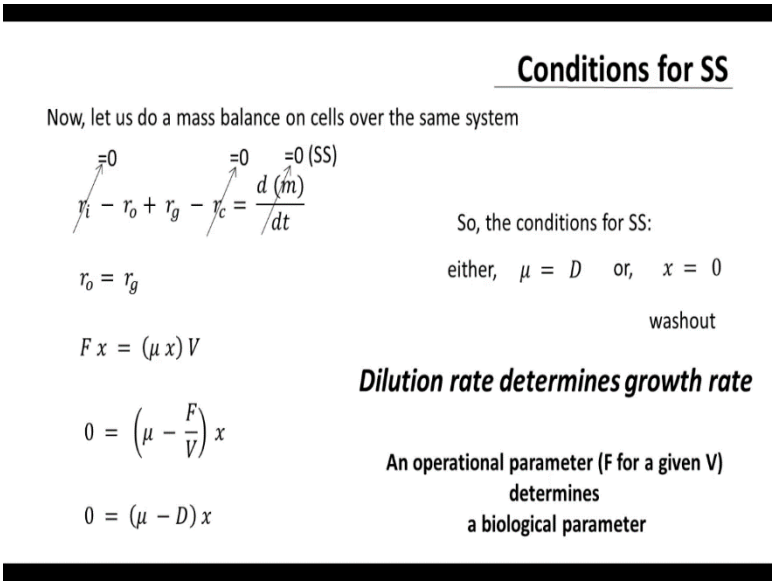


Now, let us do a mass balance on cells over the same system, earlier it was total mass balance. Now we are going to do a mass balance on cells. This is the original equation,

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

there is no input on cells it is a sterile feed, into the system therefore, r i is zero. There is no consumption of cells, there is no death of cells we will assume that. So, r c goes to zero and steady state any time derivative goes to zero.

Therefore, we get

$$r_o = r_g$$

Therefore, the rate of the output must equal the rate of generation of cells. What is the rate of output? The volumetric flow rate times the cell concentration, you work this out in terms of units you will find that this is indeed mass of cells in the outlet stream, but the mass rate of cells in the outlet stream times the rate of generation which is mu x into V, mu x is the rate of generation of cells per unit volume on a volumetric basis. We need

to multiply it by the volume to get on a mass basis, so

$$F\,x\; =\; (\mu\, x)\, V$$

So, if we transpose this equation and take x common out, you will get

$$0\; =\; (\mu\, x)\, V\; - Fx$$

$$0\; =\; \left(\mu\; -\; \frac{F}{V}\right)\, x$$

Replace F /V with D, the dilution rate, the number of reactor volumes processed per unit time.

$$0\; =\; (\mu\; -\; D)\, x$$

So, if the product of these two terms equals zero then that is possible only if any of these terms is zero or if both the terms are zero.

Either

$\left(\mu\; -\; D\right)\; =$ 0 or x= 0 or both

Only under those conditions will the equation be valid.

Now, let us look at these two conditions, what does x = 0 mean? x is what? That is the cell concentration in the outlet stream, when x equals zero means there are no cells coming out in the outlet stream which is called a washout condition. It can actually happen in the case of a chemostat operation there might be no cells that are coming out and the reactor could be a steady state even under those conditions, but it is a useless condition. We will not be able to operate effectively under those conditions you know it is non-productive having no cells in the outlet so, this is the condition for steady state this is actually called the washout condition.

Now, if we look at this mu equals d it says something very profound. Although it is a very simple equation here it says that the dilution rate determines the growth rate that is very profound because dilution rate is F/V. How do we change the dilution rate for a given reactor? For a given reactor the volume is fixed just by changing the flow rate the flow rate is under our control we change the flow rate of the pump the flow rate will change. So, just by changing the flow rate, we are able to change the specific growth rate the specific growth rate as you know is a biological parameter right. So, by changing an operational parameter which is the dilution rate by tweaking a knob, we are able to have a control over the growth rate of the organism which is a biological parameter that is something very, very profound here. So, an operational parameter F for a given V determines a biological parameter that is the significance of this equation. So, that will happen under steady state conditions of operation of a chemostat. So, if you want the cells to grow at a higher rate you just have to increase the flow rate and the cells will grow at a higher rate and till a certain point.

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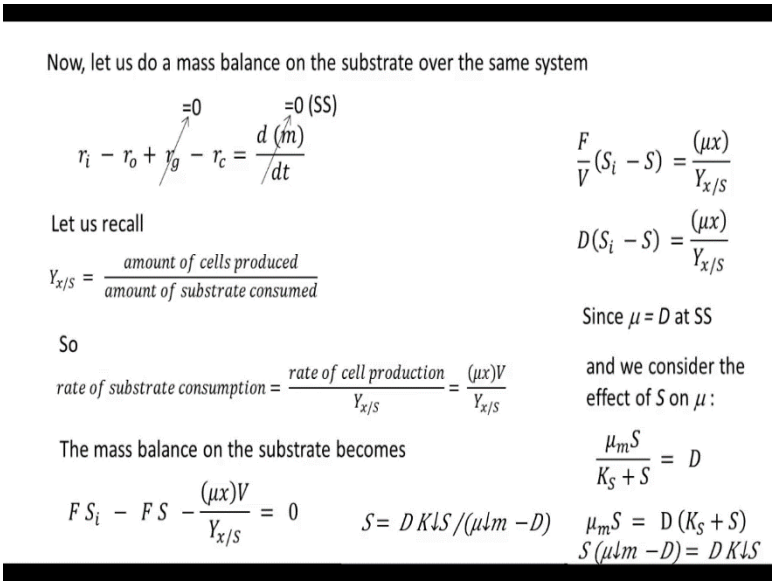


We did total mass, we did cells. Now, let us do a mass balance on the substrate over the same system same balance total.

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

In the complete balance equation, here we are going to focus on the substrate. There is an input, there is an output of the substrate. In these two streams, there is no generation of substrate, there of course is consumption and there is no accumulation because it is a steady state. All the time derivatives are zero.

$$r_i - r_o - r_c = 0$$

Let us recall that Y x/S is the amount of cells produced divided by the amount of substrate consumed.

$$Y_{x/S} = \frac{amount\ of\ cells\ produced}{amount\ of\ substrate\ consumed}$$

Why are we doing this? The input rate we can write in terms of the flow rate and the substrate concentration in the feed whereas, here we do not really have a handle on the growth rate on the consumption rate of the substrate whereas, we have a handle on the growth rate of cells. So, we are using something to represent the consumption rate of the substrate in terms of the growth rate and we all know we have already used this in a problem, the yield coefficient Y x/S can do that. Let us see how we can do that. So, the rate of substrate consumption is nothing but the rate of cell production divided by Y x/S, Y x/S is amount of cells produced by amount of substrate consumed, we are looking at rate of substrate consumed. So, let us say we divide by time above and below we get rate of cells produced by rate of substrate consumed take it to the other side the rate of substrate consumed is rate of cells produced by Y x/S, that is what is given here. Now, what is rate of cell production? It is $(\mu x)V$,

$$rate\ of\ substrate\ consumption = \frac{rate\ of\ cell\ production}{Y_{x/S}} = \frac{(\mu x)V}{Y_{x/S}}$$

So, the mass balance on the substrate becomes

$$F\,S_i \;-\; F\,S \;-\frac{(\mu x)V}{Y_{x/S}} \;=\; 0$$

Note that we derived that Fi = $F_0$ = F.

So, if we consolidate the terms on appropriately, we have

$$\frac{F}{V}(S_i \;- S) \;= \frac{(\mu x)}{Y_{x/S}}$$

We know that $\frac{F}{V} = D$

Thus the eqution becomes $D\left(S_i \;- S\right) \;= \frac{(\mu x)}{Y_{x/S}}$

Since μ = D at steady state

And we consider the effect of S on μ, i.e. $\frac{\mu_m S}{K_S + S} \;=\; \mu \;=\; D$

$$\mu_m S \;=\; \mathrm{D}\,(K_S + S)$$

$$S\,(\mu_m \;- D) \;=\; D\,K_S$$

$$S \;=\; \frac{D\,K_S}{(\mu_m \;- D)}$$

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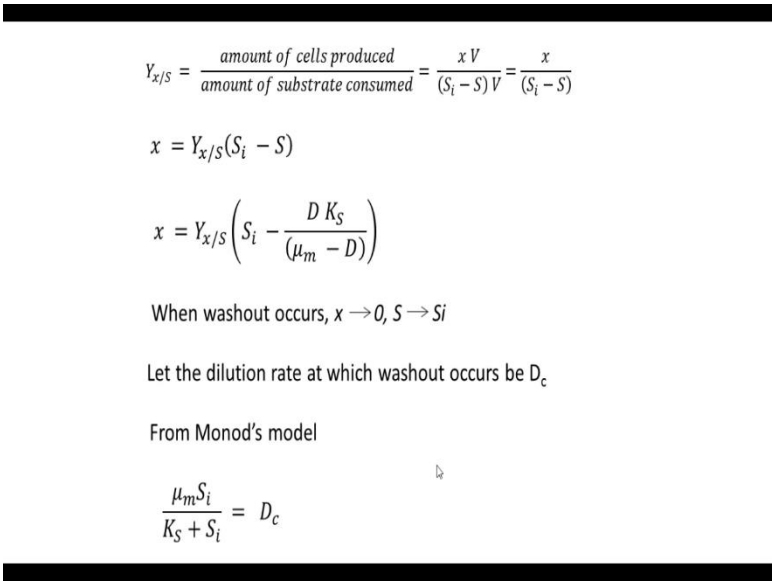


So,

$$Y_{x/S} = \frac{amount\ of\ cells\ produced}{amount\ of\ substrate\ consumed} = \frac{x\,V}{(S_i - S)\,V} = \frac{x}{(S_i - S)}$$

Thus $x = Y_{x/S}(S_i - S)$ ,

Substitute for S,

$$x = Y_{x/S}\left(S_i - \frac{D\,K_S}{(\mu_m - D)}\right)$$

When the washout occurs x →zero and there should be no change in substrate concentration from the inlet because it is not being consumed for the production of cells maybe little bit is taken up for maintenance, but that may not be very apparent. So S→Si

Let the dilution rate at which washout occurs be Dc. While we are going through all these things let us also recall why we are doing these things? We are looking at conditions for the steady state operation. Objective of our analysis is to obtain conditions

for steady state operation, to obtain the critical dilution rate the maximum operable dilution rate that is what we are looking at now through a lot of algebra. When we are doing the algebra it is good not to lose sight of this, that is why we need to remind ourselves is to what we are doing now. Let us get back, let the dilution rate at which washout occurs be Dc or the critical dilution rate.

From the Monod model

$$\frac{\mu_m S}{K_S + S} = \mu$$

Since μ = D = Dc here and S→Si, the equation can be written as

$$\frac{\mu_m S_i}{K_S + S_i} = D_c$$

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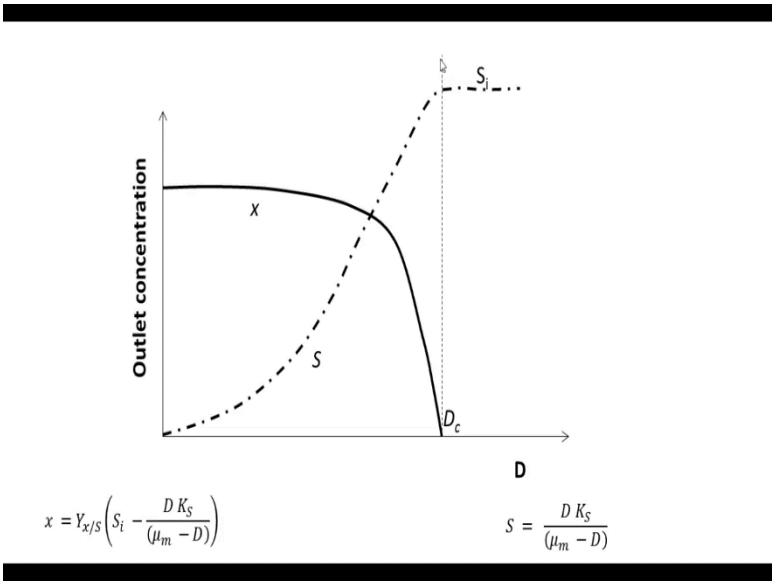
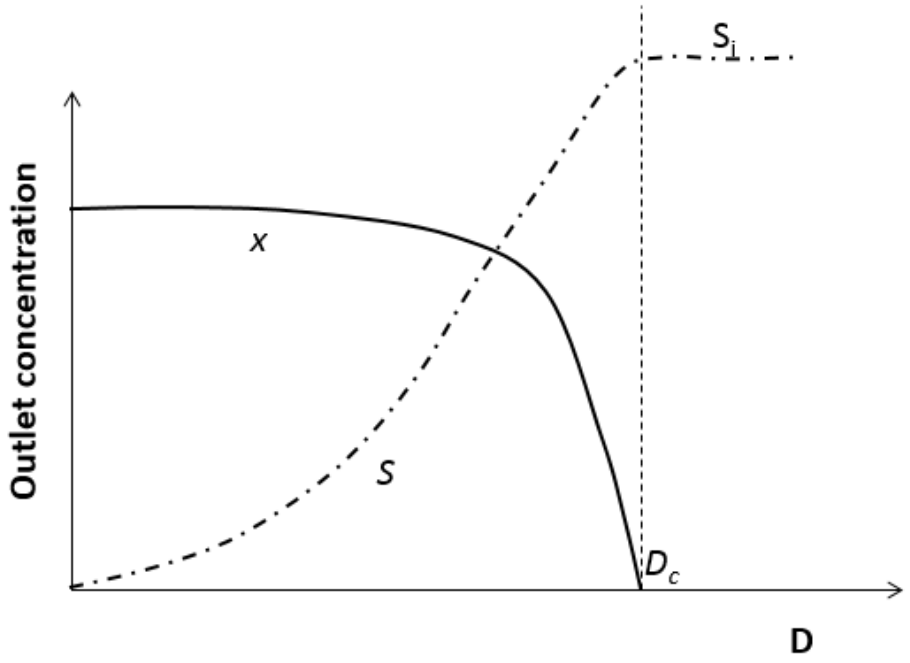



And if we plot the outlet concentration verses the dilution rate, it is this expression you know we are plotting the outlet concentration of cells which is given by this equation.

$$x = Y_{x/S}\left(S_i - \frac{D\,K_S}{(\mu_m - D)}\right)$$

If we convert this into a graph it is going to look something like this




The drop is very steep and closer to the Dc. At the critical dilution rate actually there are no cells that are present in the outlet the cell concentration is zero and from the expression that we derived

$$S = \frac{D\,K_S}{(\mu_m - D)}$$

It reaches the substrate concentration in the inlet at the critical dilution rate. So, this is the range at which you can operate a chemostat. After critical dilution rate, there will be washout, there will be no cells and there will be no conversion of the substrate, there is no point in operating the chemostat there.

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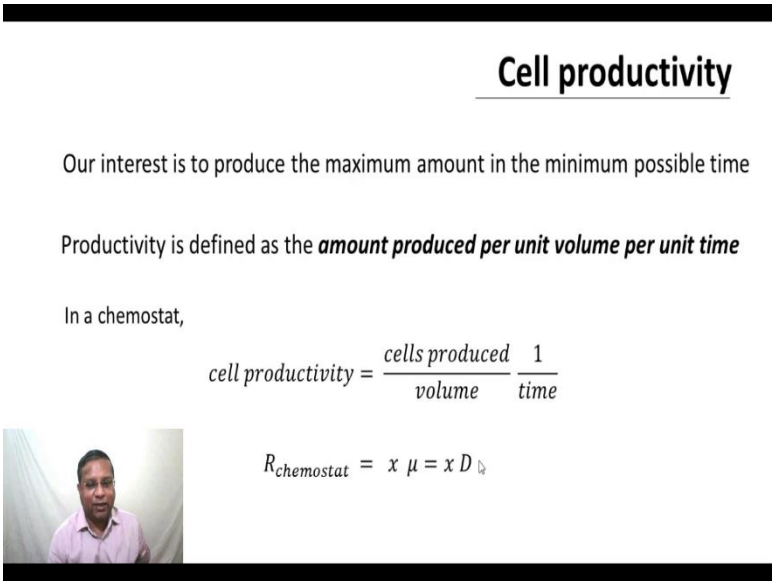


Now, the third objective if you recall we were going to compare the cell productivities of the chemostat with that of a batch bioreactor. To do that, let us see what the cell productivities are? Our interest is to usually general interest from an industry is to produce the maximum amount in the minimum possible time. The productivity is actually defined for that purpose, it is the amount produced per unit volume per unit time. In a chemostat the cell productivity is therefore, the amount of cells produced per unit volume per unit time if the cells happened to be the product.

$$cell\ productivity = \frac{cells\ produced}{volume}\ \frac{1}{time}$$

Even otherwise the cells are producing a product you would also like to maximize the cell productivity because that would directly translate to the increase in production of the molecule inside the cell, more the number of factories more the product of interest too. Therefore, we are looking at cells themselves. If we represent the chemostat productivity by R of a chemostat it would be

$$R_{chemostat} = x\ \mu = x\ D$$

Since the cells produced by unit volume is x, one by time is μ.

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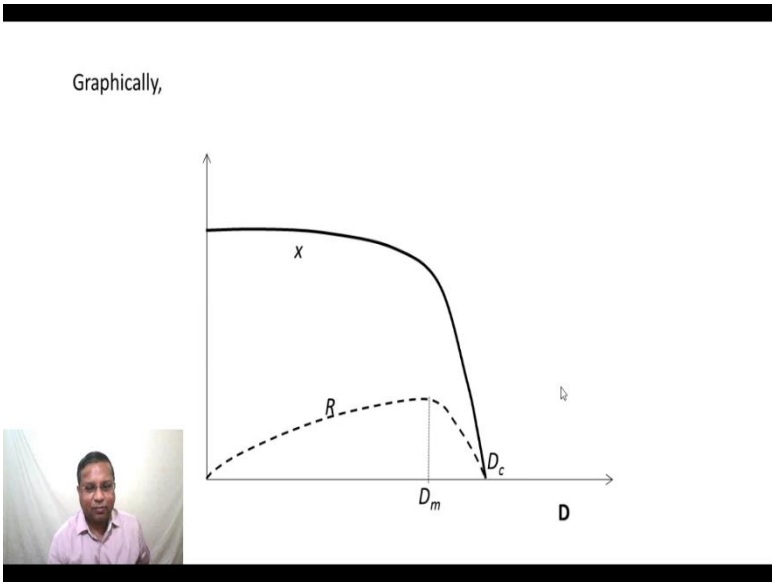
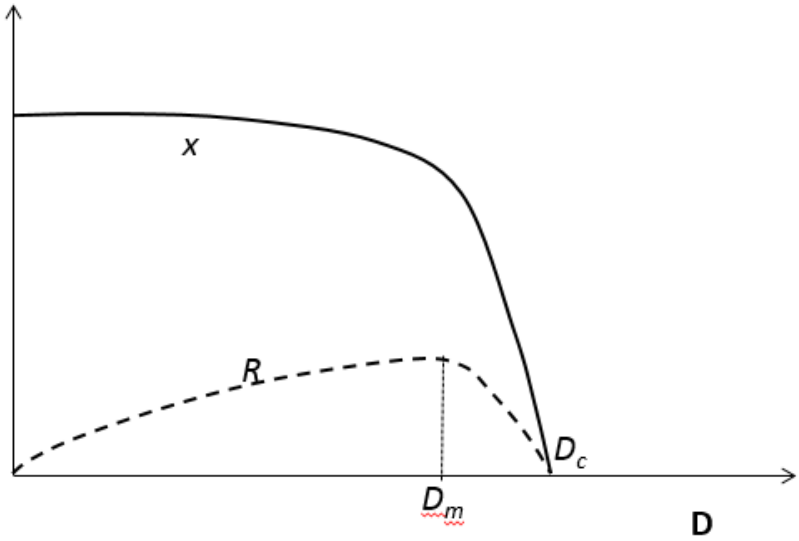



And graphically, we have



The cell concentration variation with d was as we have already seen. The productivity will go on increasing till it reaches a maximum productivity at Dm and then it will actually drop till the point of the critical dilution rate where it becomes zero. Where the cells are coming out, the productivity needs to be zero there.

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Let us find the dilution rate ($D_m$) at which cell productivity ($R$) is maximum

Let us recall that for a function, $R = f(D)$ at the maxima,

$$\frac{dR}{dD} = 0; \quad \frac{d^2R}{dD^2} < 0$$

Here,

$$R = D\,x = D\left[Y_{x/S}\left(S_i - \frac{D\,K_S}{\mu_m - D}\right)\right] = DY_{x/S}S_i - Y_{x/S}\frac{D^2K_S}{\mu_m - D}$$

$$\frac{dR}{dD} = Y_{x/S}S_i - Y_{x/S}\frac{(\mu_m - D)DK_S - D^2K_S(-1)}{(\mu_m - D)^2} = 0$$

$$Y_{x/S}S_i - Y_{x/S}\frac{\mu_m DK_S - D^2K_S + D^2K_S}{(\mu_m - D)^2} = 0$$

Let us find out the dilution rate Dm at which the cell productivity r is maximum. We are interested in maximum productivity; let us compare maximum productivities possible.

For a function, we know the conditions for a maximum, mathematically.

For example, for a function $R = f(D)$, then at the maxima

$$\frac{dR}{dD} = 0; \quad \frac{d^2R}{dD^2} < 0$$

Cell productivity R can be given by
$R = \mu x$
In a chemostat, $\mu = D$
Therefore $R = Dx$
Substituting for x

$R = DY_{\frac{x}{s}}(Si - S)$

Substituting for S in terms of D

$R = DY_{\frac{x}{s}}(Si - (\frac{DK_s}{\mu_m - D}))$ …….. (1)

It is known that for a function, $R = f(D)$,

The conditions for maxima are

$\frac{dR}{dD} = 0$ and $\frac{d^2R}{dD^2} = 0$

Therefore for maximum cell productivity

$\frac{dR}{dD} = 0$

Substituting for R from (1)

$$\frac{d\,(DY_{\frac{x}{s}}(Si-(\frac{DK_s}{\mu_m-D}))}{dD} = 0$$

$$\frac{d}{dD}\left(DY_{\frac{x}{s}}\,Si - \frac{D^2Y_{\frac{x}{s}}K_s}{\mu_m - D}\right) = 0$$

$$\frac{d}{dD}\left(DY_{\frac{x}{s}}\,Si\right) - \frac{d}{dD}\left(\frac{D^2Y_{\frac{x}{s}}K_s}{\mu_m - D}\right) = 0$$

$$Y_{\frac{x}{s}}\,Si - Y_{\frac{x}{s}}\,K_s\left(\frac{2D(\mu_m - D) - D^2(-1)}{(\mu_m - D)^2}\right) = 0$$

$$Si - K_s\left(\frac{2D\mu_m - D^2}{(\mu_m - D)^2}\right) = 0$$

Multiplying throughout with $(\mu_m - D)^2$

$Si\,(\mu_m - D)^2 = K_s(\,2D\mu_m - D^2)$

$\mu_m{}^2 + D^2 - 2D\mu_m = \frac{K_s(\,2D\mu_m - D^2)}{Si}$

$D^2\left(1 + \frac{K_s}{Si}\right) - 2D\mu_m\left(1 + \frac{K_s}{Si}\right) + \mu_m{}^2 = 0$

Which is a quadratic equation in D,

Solving for D,

$$D = \frac{2\mu_m \pm \left(4\mu_m{}^2 - \frac{4\mu_m{}^2}{\left(1 + \frac{K_s}{Si}\right)}\right)^{0.5}}{2}$$

$$D = \mu_m \left(1 - \left(1 - \frac{1}{\left(1 + \frac{K_s}{Si}\right)}\right)^{0.5}\right)$$

$$D = \mu_m \left(1 - \left(1 - \frac{Si}{Si + K_s}\right)^{0.5}\right)$$

$$D = \mu_m \left(1 - \left(\frac{K_s}{K_s + Si}\right)^{0.5}\right)$$

Therefore dilution rate Dm, at which maximum cell productivity can be obtained is given by

$$D_m = \mu_m \left(1 - \left(\frac{K_s}{K_s + Si}\right)^{0.5}\right)$$

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$$Y_{x/S} \frac{\mu_m D K_S}{(\mu_m - D)^2} = Y_{x/S} S_i$$

$$\mu_m D K_S = S_i (\mu_m - D)^2$$

$$\frac{\mu_m D K_S}{S_i} = {\mu_m}^2 - 2\mu_m D + D^2$$

$$D^2 - \mu_m \left(\frac{K_S}{S_i} + 2\right) D + {\mu_m}^2 = 0$$

This is a quadratic equation in *D*.
Solving it (after a lot of algebra), we get

$$D_m = \mu_m \left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)$$

Then, to ensure a maximum and not a minimum, we need to verify that

$$\frac{d^2R}{dD^2} < 0$$

When

$$K_S \ll S_i, \quad \left(\frac{K_S}{K_S + S_i}\right) \to 0$$

$$D_m \approx \mu_m$$

And that is what we have here; this is just transposed to take the other term one term to the other side. Now, we can cancel out the Y x S and again represent it appropriately multiply both sides by mu m minus D squared we get this, then we get the semblance of quadratic equation in D mu m D K S by S i equals, if you expand this a minus b squared

is a squared To ensure that is the maximum, not a minimum we actually need to check whether the second derivative is less than zero.

$$\frac{d^2R}{dD^2} < 0$$

If $K_S \ll S_i$, $\left(\frac{K_S}{K_S + S_i}\right) \rightarrow 0$

Then,

$$D_m = \mu_m \left(1 - \left(\frac{K_s}{K_s + Si}\right)^{0.5}\right) = \mu_m (1 - 0) = \mu_m$$

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**Cell concentration at $D_m$**

If $x_m$ is the cell concentration obtained when the productivity is maximum, then we can get an expression for $x_m$ by substituting the relevant variables in the equation for it. We know

$$x_m = Y_{x/S}\left(S_i - \frac{D_m K_S}{(\mu_m - D_m)}\right)$$

Substituting the expression for $D_m$ into the above, we get

$$x_m = Y_{x/S}\left(S_i - \frac{\left(\mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right) K_S}{\left(\mu_m - \mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)}\right)$$

.

Let us look at the cell concentration at Dm, if x m is the cell concentration obtained when the cell productivity is maximum then we can get an expression for xm by substituting the relevant variables in the equation. The corresponding variables if you substitute in the expression for x m, we should get the appropriate expression. We know that x m is the cell concentration at the maximum productivity. So, all the other variables should also correspond to the maximum productivity variables. D should actually be D m here, the rest are the same

$$x_m = Y_{\frac{x}{S}}\left(S_i - \frac{D_m K_S}{(\mu_m - D_m)}\right)$$

So, that there is no change, here D has become D m, here $K_S$ is the constant, $\mu_m$ is constant and D has become Dm here. So, substituting the expression for Dm into the above we get:

$$x_m = Y_{x/S}\left(S_i - \frac{\left(\mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)K_S}{\left(\mu_m - \mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)}\right)$$

This is the expression for the maximum cell concentration.

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This can be simplified (first few steps)

$$x_m = Y_{x/S}\left(S_i - \frac{\left(\mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)K_S}{\mu_m\left(1 - \left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)}\right) \qquad Let\ A = \left(\frac{K_S}{K_S + S_i}\right)^{0.5} \qquad Y_{x/S}\left(S_i - \frac{(1-A)K_S}{A}\right)$$

$$Y_{x/S}\left(\frac{AS_i - K_S + AK_S}{A}\right) \qquad Y_{x/S}\left(\frac{A(S_i + K_S) - K_S}{A}\right) \qquad \text{... to finally get}$$

$$x_m = Y_{x/S}\{(S_i + K_S) - (K_S(K_S + S_i))^{0.5}\} = Y_{x/S}(K_S + S_i)\left\{1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right\}$$

When $K_S \ll S_i$, $\left(\frac{K_S}{K_S + S_i}\right) \to 0$ $\qquad x_m = Y_{x/S} S_i$

$$R_m = D_m x_m = \mu_m\left(Y_{x/S} S_i\right)$$

This can be simplified the first few steps are shown here, this is the starting expression

$$x_m = Y_{x/S}\left( S_i - \frac{\left(\mu_m\left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right) K_S}{\mu_m\left(1 - \left(1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right)\right)}\right)$$

For simplicity, $Let\ A = \left(\frac{K_S}{K_S + S_i}\right)^{0.5}$

Then $x_m$ becomes

$$x_m = Y_{x/S}\left(S_i - \frac{(1 - A)K_S}{A}\right)$$

$$= Y_{\frac{x}{S}}\left(\frac{AS_i - K_S + AK_S}{A}\right)$$

$$= Y_{x/S}\left(\frac{A(S_i + K_S) - K_S}{A}\right)$$…….. to finally get

$$x_m = Y_{x/S}\left\{(S_i + K_S) - \left(K_S(K_S + S_i)\right)^{0.5}\right\} = Y_{x/S}\,(K_S + S_i)\left\{1 - \left(\frac{K_S}{K_S + S_i}\right)^{0.5}\right\}$$

This is the expression for the maximum cell concentration.

$When\ K_S \ll S_i$, then $\left(\frac{K_S}{K_S + S_i}\right) \to 0$, therefore $x_m\ becomes$

$$x_m = Y_{\frac{x}{S}}\,S_i$$

Consequently the productivity Rm would be

$$R_m = D_m x_m = \mu_m\left(Y_{x/S}\,S_i\right)$$

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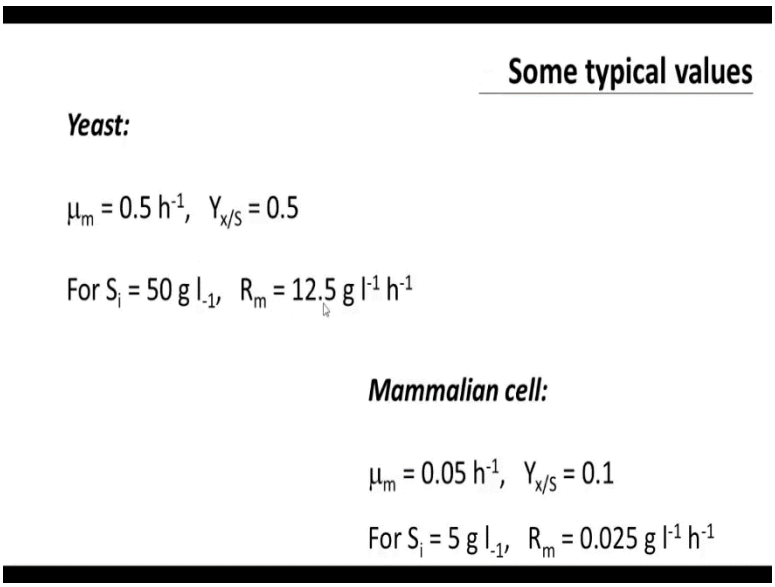


Let us look at some typical values to get an idea*:*

In yeast, typical values are

$\mu_m = 0.5\ h^{-1}$, $Y_{\frac{x}{s}} = 0.5$

So for an inlet substrate concentration Si = 50 $gl^{-1}$, the maximum productivity would be $R_m = 12.5\ gl^{-1}h^{-1}$

Similarly for mammalian cells, the typical values are $\mu_m = 0.05\ h^{-1}$, $Y_{\frac{x}{s}} = 0.1$, thus for Si = 5 $gl^{-1}$, $R_m = 0.025\ gl^{-1}h^{-1}$

So, here you get 12.5 gram per liter per hour as a typical productivity in yeast and only 0.025 gram per liter per hour in mammalian cells. You see the change in the magnitudes.

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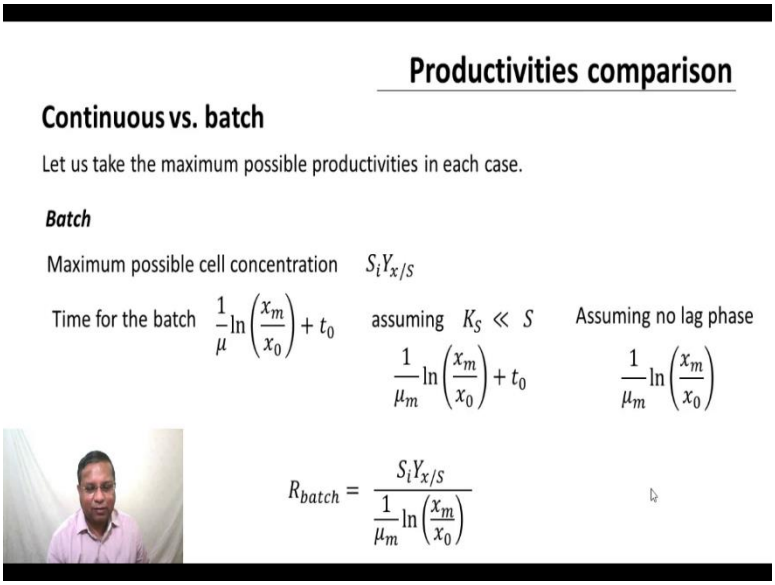


Now, let us compare productivities of continuous and batch operations. Let us take the maximum possible productivity in each case and let us see what the ratio. For the batch, we know the maximum possible cell concentration is $S_i Y_{x/S}$.

Time for the batch is

$$t = \frac{1}{\mu}\ln\left(\frac{x_m}{x_0}\right) + t_0$$

Assuming no lag phase, we have

$$t = \frac{1}{\mu}\ln\left(\frac{x_m}{x_0}\right)$$

When we assume that $K_S \ll S$, $\mu = \mu_m$

I am doing all this to get an idea of the ratio of productivities if the lag time is significant. We can always add them for comparison; I am trying to do that on a generic basis. So, I am taking the simplest case that is possible, but which is still representative.

So, the productivity of batch is the maximum possible cell concentration divided by the time that it takes for the batch, i.e. the amount produced per unit volume per unit time.

$$R_{batch} = \frac{S_i Y_{x/S}}{\frac{1}{\mu_m} \ln\left(\frac{x_m}{x_0}\right)}$$

This is the productivity expression for a batch starting from $x_0$ going up to $x_m$.

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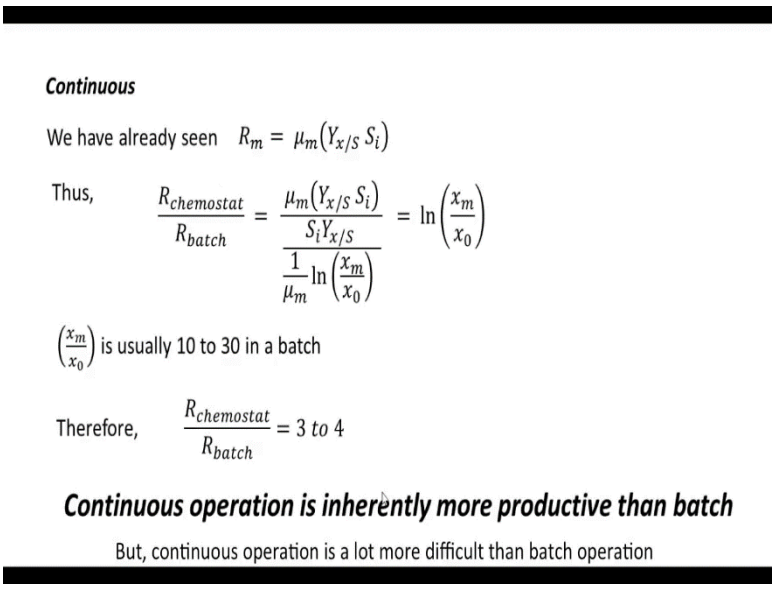


Now, for the chemostat case we already seen that the maximum productivity is

$$R_m = \mu_m(Y_{x/S} S_i)$$

Therefore, the ratio of the maximum productivities of a chemostat to that of batch is:

$$\frac{R_{chemostat}}{R_{batch}} = \frac{\mu_m(Y_{x/S} S_i)}{\frac{S_i Y_{x/S}}{\frac{1}{\mu_m}\ln\left(\frac{x_m}{x_0}\right)}} = \ln\left(\frac{x_m}{x_0}\right)$$

Typically in a batch situation you have the maximum cell concentration being about 10 to 30 fold the initial cell concentration.

Since $\left(\frac{x_m}{x_0}\right)$ is usually 10 to 30 in a batch

$$\frac{R_{chemostat}}{R_{batch}} = 3\ to\ 4$$

Thus the ratio of the maximum productivities of a chemostat to that of a batch is three to four. This says that, inherently, the chemostat is three to four times more productive than a batch reactor. So, this is by the very nature of it. This is also profound kind of insight that we get from this analysis. The chemostat is three to four times more productive than a batch usually speaking.

So, in industry we can expect to be three to four times more productive by switching to a continuous operation, but operating in a continuous mode requires a lot more effort than operating in a batch mode that is the reason why industries prefer a batch except if it is completely automated in so and so forth, then some industries have switch to continuous might want to internalized this by going through this again we have shown that a continuous bioreactor is inherently three to four times more productive than a batch bioreactor.

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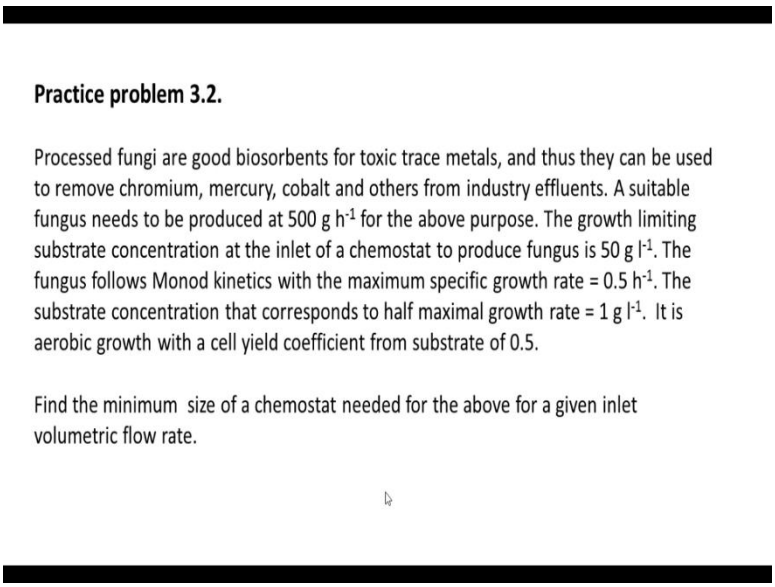


Let us see a practice problem, I will assign this problem and solve it in the next lecture. We will continue after this problem also, this fed batch operation which I would like to complete as a part of this particular lecture itself. The practice problem reads as follows processed fungi are good biosorbents for toxic trace metals and thus they can be used to remove chromium, mercury, cobalt and other heavy metals from industry effluents a suitable fungus needs to be produced at 500 grams per hour. For the above purpose, the growth limiting substrate concentration at the inlet of a chemostat to produce fungus is 50 grams per liter, the fungus follows Monod kinetics and the maximum specific growth rate is 0.5 hour inverse the substrate concentration that corresponds to the half maximal growth rate is 1 gram per liter it is aerobic growth with a cell yield coefficient from substrate of 0.5 find the minimum size of a chemostat needed for the above for a given inlet volumetric flow rate. So, why do not you try this out? We know of course, see the solution in the next lecture.

Now let us continue.

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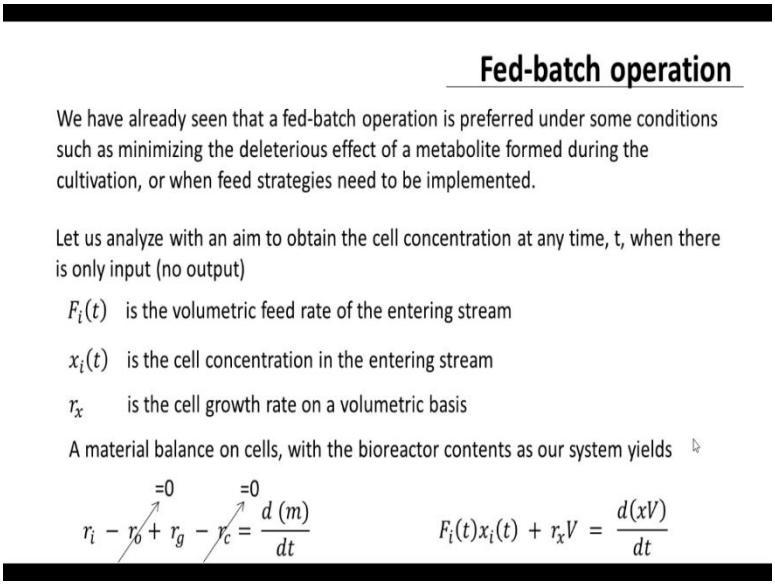


The fed batch operation. How to analyze the fed batch operation? As you recall, fed batch is nothing but intermittent addition, intermittent removal, together or one at a time. There is a reason for doing the fed batch operation. In fact, that is the most common mode in an industry. We have already seen that the fed batch operation is preferred under some conditions such as minimizing the deleterious effect of a metabolite formed during the cultivation or when some special feed strategies need to be implemented. This is one of the situations where one would prefer a fed batch operation and let us analyze this operation with an aim to obtain the cell concentration at any time t, when there is only input. I am just taking one simple case to analyze, to just start you on the analysis, it can get a lot more complex as long as you know the functionality you would be able to solve, you will be able to get expressions and solve them towards your needs later. So, in this case there is only input, there is no output. The let us say that Fi as a function of t is the volumetric feed rate of the entering stream and $x_i(t)$ is the cell concentration in the entering stream, just for generalization. It is not a sterile feed and $r_x$ is the cell growth rate on a volumetric basis.

If we do a material balance on cells with the bioreactor contents or the bioreactor broth as a system, this is the overall balance equation.

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

There is no output, there is only input. Let us assume there is no consumption of cells. This is not a steady state, so $\frac{d(m)}{dt}$ cannot go to zero.

Therefore $r_i + r_g = \frac{d(m)}{dt}$

Substituting for the terms,

$$F_i(t)x_i(t) + r_xV = \frac{d(xV)}{dt}$$

$$F_i(t)x_i(t) + r_xV = x\frac{d(V)}{dt} + V\frac{d(x)}{dt} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (i)$$

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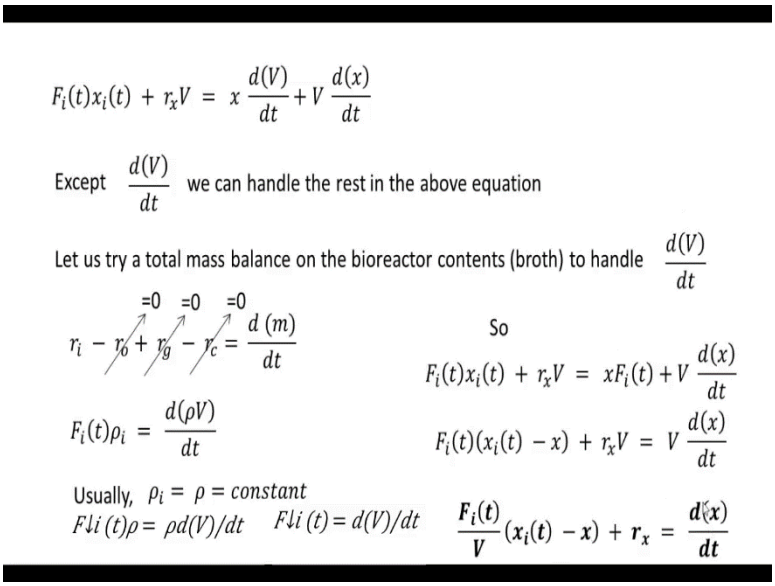


Now, we no longer have that niceness of something being constant, all the terms are there. So, Fi is a function of t, $x_i$ is a function of t . V is not a constant right. So, all these

terms are still there. Except the term $\frac{d(V)}{dt}$, we can handle the rest of the terms. We know the inlet feed rate as a function of time, we know the inlet cell concentration as a function of time, we know rate of cell formation and we know the volume of the bioreactor. So, this is the only term that we need to handle to get a handle on this equation.

To do that, let us try a total mass balance on the bioreactor broth.

$$r_i - r_o + r_g - r_c = \frac{d(m)}{dt}$$

In the total mass balance, there is no output, no generation of mass, no consumption. So all these terms go to zero and therefore,

$$r_i = \frac{d(m)}{dt}$$

Substituting,

$$F_i(t)\rho_i = \frac{d(\rho V)}{dt}$$

Usually the inlet and outlet densities are the same and a constant therefore, we can take them out of the derivative.

$$F_i(t)\rho = \rho\frac{d(V)}{dt}$$

$$F_i(t) = \frac{d(V)}{dt}$$

Substituting for $\frac{d(V)}{dt}$ in (i), we have

$$F_i(t)x_i(t) + r_xV = xF_i(t) + V\frac{d(x)}{dt}$$

$$F_i(t)(x_i(t) - x) + r_xV = V\frac{d(x)}{dt}$$

$$\frac{F_i(t)}{V}(x_i(t) - x) + r_x = \frac{d(x)}{dt}$$

If these functionalities are known, then we can solve this to get the cell concentration profile in the fed batch bioreactor. We will stop here for this module.

In fact, we have completed this module on bioreactor analysis, the common modes of operation. We initially saw the batch bioreactor, analyzed them, analyzed the batch bioreactor towards getting some useful estimates and then we looked at the continuous operation, a problem has been assigned, please do that, and finally, we looked briefly at how to handle fed batch operation.