

**Bioreactor Design and Analysis**  
**Dr. Smita Srivastava**  
**Department of Biotechnology**  
**Indian Institute of Science – Madras**

**Lecture 12**  
**Design of Fed Batch Bioreactors - Practice Problems - Part 3**

(Refer Slide Time: 00:17)



**Problem 2**

*Pseudomonas AM-1* cells are cultured to high density for production of clavulanic acid. The reactor used is a stirred tank that initially contains 100 litres of medium. The maximum specific growth rate of the culture is  $0.18 \text{ day}^{-1}$  and the yield of biomass from substrate is  $0.5 \text{ g/g}$ . The concentration of growth-limiting substrate in the medium is  $3\% \text{ (w/v)}$ . The reactor is inoculated with  $1.5 \text{ g/L}$  of cells and operated in batch until the substrate is virtually exhausted; medium flow is then started at a rate of  $4 \text{ L/day}$ . Fed-batch operation is carried out for 40 days under quasi-steady-state conditions.

(a) Estimate the batch culture time and the final biomass concentration achieved after the batch culture period.

(b) What is the final mass of cells in the reactor?

(c) The fermenter is available 275 days per year with a downtime between runs of 24 h. How much cell biomass is produced annually?



Let us check out problem number 2 *Pseudomonas am1* cells are cultured to high density for production of Clavulanic acid. The reactor used is a stirred tank that initially contains 100 liters of medium. So we have initial volume as 100 liters the maximum specific growth rate of the culture is  $0.18 \text{ day}^{-1}$  and the yield of biomass from substrate is  $0.5 \text{ grams per gram}$ . So we have  $Y_{X/S}$  given to us as  $0.5$ .

Let us try to list down what is given,  $\mu_{\max}$  is given to us as  $0.18 \text{ day}^{-1}$  and  $V_0$  is hundred liters at  $t = 0$  hour the concentration of growth limiting substrate in the medium is  $3\%$ . So the feed concentration is  $30 \text{ grams per liter}$  the reactor is inoculated with  $1.5 \text{ grams per liter}$  of cells. So it has been initially inoculated with these many cells concentration and operated in batch until the substrate is virtually exhausted.

So like in the previous problem it has been the batch has started with  $1.5 \text{ grams per liter}$  of  $X$  till all the  $S$  is consumed medium is then started after all the  $S$  is consumed the batch is converted to fed batch starting the feed at a flow rate of  $4 \text{ liters per day}$ . Fed batch operation is then carried out until 40 days at quasi steady state. So when we convert the batch to fed

batch when nearly S is 0 at this point your X max which is Y X by S into S i this is the biomass concentration which is continued for another 40 days.

Estimate the batch culture time and the final biomass concentration achieved at the batch culture period. So this should be straightforward for you all? So batch culture time assuming this culture is growing at its maximum specific growth rate. So we can determine the batch time using the expression given here.

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**Solution**

(a) The initial substrate concentration  $S_0 = 3\%$  (w/v) = 3 g per 100 ml = 30 g/L

The batch culture time to achieve  $S_f = 0$  is determined by

$$(t)_b = \frac{1}{\mu_{max}} \ln \left[ 1 + \frac{Y_{XS}}{x_0} (S_0 - S_f) \right]$$

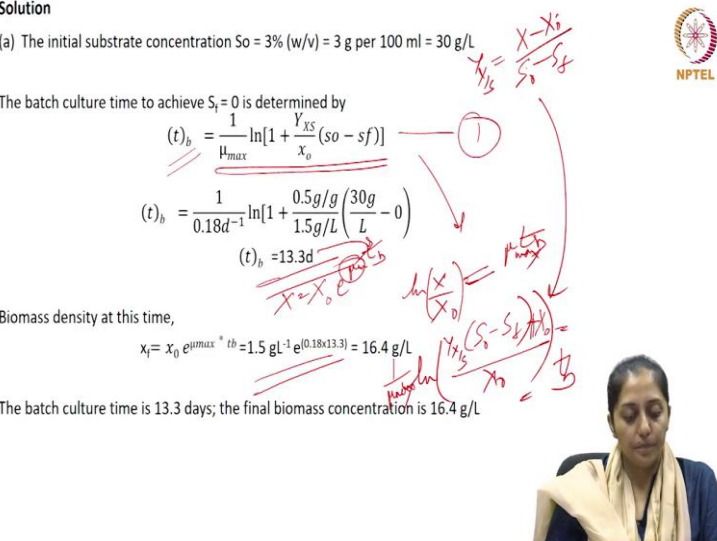
$$(t)_b = \frac{1}{0.18 d^{-1}} \ln \left[ 1 + \frac{0.5 g/g}{1.5 g/L} \left( \frac{30 g}{L} - 0 \right) \right]$$

$$(t)_b = 13.3 d$$

Biomass density at this time,

$$x_t = x_0 e^{\mu_{max} t} = 1.5 g/L \cdot e^{(0.18 \times 13.3)} = 16.4 g/L$$

The batch culture time is 13.3 days; the final biomass concentration is 16.4 g/L



The slide contains handwritten notes in red ink. At the top right, there is a diagram showing the relationship between biomass concentration (X) and substrate concentration (S). The initial biomass concentration is X<sub>0</sub> and the initial substrate concentration is S<sub>0</sub>. The final biomass concentration is X<sub>t</sub> and the final substrate concentration is S<sub>f</sub>. The relationship is shown as X - X<sub>0</sub> = Y<sub>XS</sub>(S<sub>0</sub> - S<sub>f</sub>). Below this, there is a circled '1' next to the general equation for batch culture time. The specific calculation for (t)<sub>b</sub> is shown with the values substituted. The final result is (t)<sub>b</sub> = 13.3 d. Below this, the biomass density at this time is calculated as x<sub>t</sub> = 16.4 g/L. In the bottom right corner, there is a small video inset of a woman with a yellow shawl, who appears to be the instructor, looking at a document.

I hope you can understand how we have reached to this. So we know that  $\ln X$  by  $X_0$  is equals to  $e$  to the power of  $\mu t$  where here  $\mu$  stands for  $\mu_{max}$  and  $t$  will be your batch culture time and  $X$  is nothing but  $Y_{XS} (S_0 - S_f) + X_0$ ,  $S_f$  is final. So if we expand this sorry  $\mu$  times because it is  $\ln X$  by  $X_0$ . So this will be equal to  $\mu$  times  $t_b$ . So this has come from  $\ln X$  by  $X_0$  is equals to  $\mu t$  where  $\mu$  is equals to  $\mu_{max}$  and  $t$  is your  $t_b$  which is batch time.

So if we expand this further we will be able to determine the batch time as shown here in this equation your  $X$  will be  $X_0 + Y_{XS} (S_0 - S_f)$  correct. So we know that  $Y_{XS}$  is equal to for batch  $S_0$  minus sorry  $X$  minus  $X_0$  divided by  $S_0$  minus  $S_f$  so from here is the numerator. So now we can find the value of the batch time which comes out to be 13.3 days and if you want to determine now the biomass final concentration it will be  $X$  is equals to  $X_0 e^{\mu_{max} t_b}$ .

So we know now the value of batch time we know  $\mu_{max}$  and this is what has been done here  $X_0$  is also known so we can find now the final biomass concentration at the end of the batch what is the final mass of the cells in the reactor after 40 days.

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(b) The mass of cells at the start of fed-batch operation is equal to the final batch cell concentration multiplied by the batch medium volume:

$$X_0 = x_f V = 16.4 \text{ g/L} * (100 \text{ L}) = 1640 \text{ g}$$


The final mass of cells after 40 d fed-batch culture

$$X = X_0 + (Y_{XS} S_f) F t_{fb}$$

$$X = 1640 \text{ g} + (0.5 \text{ g/g}) * (30 \text{ g/L}) * (4 \text{ L/d}) * (40 \text{ d})$$

$$X = 4040 \text{ g} = 4.04 \text{ kg}$$

$$x_f V = 100 * 16.4 = 1640$$



So at the beginning of the fed batch we know the mass of the cells would be the concentration achieved multiplied by the volume, volume is given as 100 liters and the concentration which we achieved was 16.4 grams per liter. So at  $t$  is equals to 0 when feeding started the initial amount of biomass was 1640. So if you remember the expression for fed batch this is the maximum biomass concentration at which the feeding has started at quasi steady state at a feed flow rate of  $f$  and this is the time period of feeding which is now 40 days.

We substitute the value of  $f$  as 4 liters per day and your feed substrate concentration is 30 grams per liter and your  $Y_{X/S}$  given is 0.5 where  $X_0$  is the initial amount of cells at the beginning of this fed batch which is 1640 which we obtained from here. So our final biomass amount comes out to be 4040 grams at the end of 40 days of fed batch at pseudo steady state.

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(c) The mass of cells produced in each reactor run is equal to the final biomass minus the biomass used for inoculation:

Biomass produced per run =  $4040 \text{ g} - (1.5 \text{ g/L}) * (100 \text{ L}) = 3890 \text{ g} = 3.89 \text{ kg}$

The total reaction time is,

$$t_r = t_f + t_{fb} + t_{dn}$$

$$t_r = 13.3d + 40d + 1d$$



$$t_r = 54.3d$$

In one year, the number of runs carried out:

Number of runs =  $\frac{275d}{54.3d/run} = 5.06$

The total biomass produced annually is equal to the biomass produced per run multiplied by the number of runs per year

Biomass produced per year =  $3.89 \text{ kg/run} \times 5.06 \text{ runs} = 19.7 \text{ kg}$

Let us see the third part it is said that the fermenter is available for 275 days per year with a down time between runs of 24 hours. How much cell biomass is produced annually? So the total time taken to achieve this 4040 grams which is one single fed batch process would be the downtime plus the batch time plus the fed batch time. So if we calculate it is 24 hours plus 13 days 13 point something 13.3 days plus the fed batch time is 40 days.

So this is the total time for running one fed batch process and obtaining 4040 minus if we remove actual amount of biomass which we could achieve it will be 1.5 times the volume at this stage was 100 liters so actually it is  $4040 - 150$ . See this is what has been done so in actually actual amount is 3.89 kgs which we will be able to produce after running one fed batch process including the downtime.

So after these many days we will be able to produce the 0.89 kgs. So annually it has been asked. So how many runs are possible. So this is one run complete for fed batch. So, 5.06 runs are possible because 275 days it is operational. So let us assume 5 runs are possible with the biomass produced as 3.89 kg per run. So then the total amount of biomass which can be produced annually will be 19.7 kgs.

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**Problem 3**


*Lactobacillus casei* is propagated under essentially anaerobic conditions to provide a starter culture for manufacture of Swiss cheese. The culture produces lactic acid as a by-product of energy metabolism. The system has the following characteristics:

$Y_{xs} = 0.23 \text{ kg kg}^{-1}$   
 $K_s = 0.15 \text{ kg m}^{-3}$   
 $\mu_{\max} = 0.35 \text{ h}^{-1}$   
 $m_s = 0.135 \text{ kg kg}^{-1} \text{ h}^{-1}$

A stirred fermenter is operated in fed-batch mode at quasi steady state with a feed flow rate of  $4 \text{ m}^3 \text{ h}^{-1}$  and feed substrate concentration of  $80 \text{ kg m}^{-3}$ . After 6 h, the liquid volume is  $40 \text{ m}^3$ .

(a) What was the initial culture volume?  
 (b) What is the concentration of substrate at quasi-steady state?  
 (c) What is the concentration of cells at quasi-steady state?  
 (d) What mass of cells is produced after 6 h fed-batch operation?

*Handwritten notes:*  
 $V = V_0 + Ft$   
 $V_0 = V - Ft$   
 $= 40 - 4 \times 6$   
 $= 40 - 24 = 16 \text{ m}^3$



Let us read the third problem. *Lactobacillus casei* is propagated under essential anaerobic conditions to provide a starter culture for manufacture of Swiss cheese. The culture produces lactic acid as a byproduct of energy metabolism. The system has the following characteristics so they have given to us the yield coefficient of X by S Monod saturation constant maximum specific growth rate and the maintenance coefficient. A stirred fermenter is operated in fed batch mode at quasi steady state with a feed flow rate of 4 meter cube per hour.

So this is nothing but  $f$  and feed substrate concentration of 80 kg per meter cube. So this is our  $S_i$ . After six hours the liquid volume is so let us assume at  $t$  is equals to 6 the liquid volume is 40 meter cube what is the initial culture volume? So if it is a constant feed flow rate system then  $V$  is equals to  $V_0 + Ft$ . So we need to determine the  $V_0$ . So it will be  $V - Ft$ ,  $V$  is known to be 40 meter cube  $F$  is 4 meter cube per hour and time is 6 hour. This comes out to be 16 meter cube.

This is what is shown here what is the concentration of substrate at quasi steady state? So now we need to determine the substrate concentration at quasi steady state.

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b) At quasi steady state and at high cell density,  $s \ll s_0$  and also applying  $D \approx \mu$  and taking maintenance substrate requirements in consideration and  $q_p = 0$ , we have

$$\frac{ds}{dt} = D(s_1 - s) - \left( \frac{\mu}{Y_{XS}} + m_s \right) x$$

$$0 = D s_1 - \left( \frac{D}{Y_{XS}} + m_s \right) x$$

Solving for  $x$ ,

$$x = \frac{D s_1}{\frac{D}{Y_{XS}} + m_s} = \frac{0.10 \text{ h}^{-1} (80 \text{ kg m}^{-3})}{\frac{0.10 \text{ h}^{-1}}{0.23 \text{ kg kg}^{-1}} + 0.135 \text{ kg kg}^{-1} \text{ h}^{-1}} = 14.0 \text{ kg m}^{-3}$$

So if you remember the substrate concentration at quasi steady state in terms of the dilution rate is given as this now we need to determine  $D$  which is  $F$  by  $V$  we have calculated we know the final volume and the volumetric flow rate. So 4 divided by 40.1 hour inverse is our dilution rate after 6 hours. So knowing the value of  $K_S$  and  $\mu_{max}$  we can find the residual substrate concentration or the steady state substrate concentration which is 0.06 kg per meter cube.

And if you see the initial substrate concentration was 80 kg per meter cube. So we can assume that the substrate concentration at steady state is nearly 0 all has been consumed before at before the steady state was reached. So if we do the substrate balance across this fed batch reactor at quasi steady state when there is endogenous metabolism present maintenance coefficient cannot be neglected.

So the first term is the rate at which the substrate is coming inside the second term is the rate at which the substrate is getting diluted. So we can assume because this is very low substrate remaining at steady state so this is nearly 0. And this term stands for the rate at which the substrate is getting consumed for biomass formation. So we need to determine this  $X$  which is your steady state biomass concentration.

Assuming steady state so there is no accumulation. If we rearrange this equation in the form of  $d$  here applying steady state equality of  $\mu$  is equals to  $D$  with biomass balance at steady state then this equation can be written only in the form of  $D$  and the given constants and the

initial substrate concentration. So your biomass concentration at pseudo steady state will be  $D S_i$  divided by this term which comes out to be 14 kg per meter cube.

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c) Mass of cells after 6 h of fed-batch operation is given by,

$$X = x V = 14.0 \text{ kg m}^{-3} (40 \text{ m}^3) = 560 \text{ kg}$$

At the start of fed-batch operation when the liquid volume is  $16 \text{ m}^3$ , if operation is at quasi-steady state, the cell concentration =  $14 \text{ kg m}^{-3}$

$$X = x V = 14.0 \text{ kg m}^{-3} (16 \text{ m}^3) = 224 \text{ kg}$$

mass of cells produced during fed-batch operation is  $(560 \text{ kg} - 224 \text{ kg}) = 336 \text{ kg}$

Handwritten notes on the slide:

- $560 - 224 = 336 \text{ kg}$
- $t = 0$
- $X_0 = 14 \times 16 = 224 \text{ kg}$

The 4th part is what mass of cells is produced after 6 hours of fed batch operation. So we need to now find the amount of cells. So if you remember the correlation if we know the final amount the concentration the steady state concentration of the biomass and we know the final volume we can determine the amount of biomass at quasi steady state after six hours of fed batch operation.

Now at the start of the operation when the liquid volume was 16 meter cube if operation is at quasi steady state the cell concentration was 14 kg per meter cube. So the initial volume also we know was 16 meter cube the initial biomass concentration was 14 kg per meter cube at the start of the fed batch. So at the beginning of  $t$  is equals to 0 the amount was present as because it is running at pseudo steady state the so the biomass concentration will remain the same.

And we know the initial volume so  $X_0$  multiplied by  $V_0$  will be the amount of so rather than let us use the same notation small  $x$  stands for concentration which remains same because it is pseudo steady state and  $V_0$  is the volume initially which was 16 meter cube. So your initial amount of biomass can be given as 14 multiplied by 16 to 24 kgs. So if the question asked is how much amount of biomass could be produced.

So actually from this initial from this final amount of biomass achieved we will reduce the amount of biomass which was present at  $t$  is equals to 0 of this fed batch. So amount of biomass which is produced at steady state run of this fed batch was for 6 hours duration six hours would be  $560 - 224, 336$  kgs.