


Bioreactor Design and Analysis
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Lecture 07
Design of Batch Bioreactors - Practice problems

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For a microbial batch bioprocess
the student will be able to



- Apply lecture 1 & 2 content to predict batch reactor kinetics
- For given culture kinetic parameters and substrate conversion, determine
 - required reactor volume
 - productivity




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Welcome back so in the last class we had learnt about the theory for how to design a batch reactor system and we will see how to find the solutions for those problems. So let us begin with the first problem can you see the problem on the slide.

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Example




Single cell protein is produced from methanol using a 1000 m³ bioreactor.

The biomass yield from substrate is 0.42 g/g, K_s is 0.7 mg/l and μ_{max} is 0.45/h.

The medium contains 4 % (w/v) methanol and a substrate conversion of 98% is desirable in the process.

For batch mode of operation, the initial cell concentration is 0.1 g/l and the down time between batches is 5 h.

- (i) Calculate the annual biomass production
- (ii) If the desired annual biomass production is to be 10000 tons/year, what should be the volume of the reactor?



Now let us read the problem single cell protein is produced from methanol using a 1000 meter cube bioreactor. So here methanol I hope you can understand will be the yes the

substrate and the volume of the bioreactor is also given to us which is 1000 meter cube. The biomass yield which is of the single cell protein from the substrate is given to us as 0.42 grams per gram the monoth saturation constant K S is also given as 0.7 milligrams per liter.

And the maximum specific growth rate is given as 0.45 hour inverse. The medium is set to contain 4% methanol. So which would mean what as it is weight by volume so this corresponds to 40 grams per liter of methanol and a substrate conversion of 0.98 is desirable in the process. So what is desired is 98% of the substrate by the end of the batch process should get consumed.

Now for batch mode of operation the initial cell concentration is 0.1 grams per liter. So this is given to us and the down time between the two successive batches is set to be 5 hours. So generally downtime means the time which is spent in harvesting then re-preparing sterilizing the reactor for the next run cleaning all that comes under the downtime till the next reactor run is ready to start. So what has been asked is first calculate the annual biomass production?

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Total mass of cells (X) produced during the batch culture

$$X = (X_f - X_0)V$$



Total time for the culture,

$$t_T = t_b + t_d$$

The number of batches carried out in one year

$$\text{No. of batches} = \frac{365 \times 24}{t_T}$$

Annual biomass production (tons/year) = X * No. of batches





So let us see how can we calculate the annual biomass production first so first we need to calculate total mass of the cells which will be produced during this patch culture. Now the total mass would be what then of course the mass of the cells which will be produced should be during the batch process will be the difference between the final and the initials biomass concentration multiplied by the volume of the batch.

Now to find out the batch time for the culture and the total time which is needed before you run the next batch will be the sum of the batch time and the downtime then only you can run the you are ready to run the second batch. So the total time which is required or which is the gap between the two batches will be the batch time + the downtime spent for the one batch effectively. So the number of batches which can be carried out in one year can be calculated as this given an equation one.

Here 365 the number of days 24 the number of hours in each day and total time given as T with the subscript capital T. So this will give us the total number of patches which can be run in an entire duration of one year now. So now the annual biomass concentration which then can be produced would be equal to so annual means in one year. So in one year then how much amount of biomass will be be able to produce the biomass produced in the one in one batch multiplied by the total number of batches which are possible in a year. So this is what is given here.

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(i) Calculating the annual Biomass

$S_i = S_0 = 4\% \text{ (w/v)} = (4\text{g per } 100\text{ml}) = 40 \text{ g/L}$

$S_f = S = .02 \times 40\text{g/L} = 0.8\text{g/L}$

(98% conversion, so only 2% substrate remains)

$X_0 = 0.1\text{g/L}$

Batch culture time,

$$t_b = \frac{1}{\mu_{\max}} \ln \left[1 + \frac{Y_{X/S}}{x_0} (s_0 - s_f) \right]$$

$= 11.35\text{h}$

$X_f = X_0 e^{\mu t_b} = 16.5\text{g/L}$

Handwritten notes:

- $\mu = \frac{\mu_{\max} S}{K_s + S}$
- $S \rightarrow K_s$
- $K_s \ll S$
- $\mu \rightarrow \mu_{\max}$
- $\left[\frac{X_0 + Y_{X/S} (S_0 - S_f)}{X_0} \right]$

Now we need to calculate the annual biomass production. so how do we do that what is given to us the initial substrate concentration which I said corresponds to 40 grams per liter of methanol. Now the final substrate concentration is also known to us because if you remember the problem it was given that 98% of the substrate is to be converted desirably. So if 98 gets consumed so 2% remains so the residual substrate concentration or the final substrate concentration in the broth would be 0.8 grams per liter as done here after 98% conversion of the substrate.

Now if the initial biomass concentration was given as 0.1 grams per liter we can calculate the batch time it is given as 11.67 hours, how? Now your batch time can be calculated as given in this equation which I have underlined on the slide any guesses how we have reached to this equation? So let us see that the culture is following Monod's kinetics. Now if you remember the problem and if you see the problem carefully the value of K_S is very very less than the initial substrate concentration which is given as in the orders of ppm.

Whereas the substrate concentration is in grams per liter the tens of which means 40 grams per liter? So if the K_S in the Monod's model becomes very, very less than the S in this equation if K_S becomes very, very less than S then we sometimes neglect K_S . So then your μ tends to μ_{max} . Now we know $Y_{x/y}$ by S is equals to $x - x_0$ divided by $S_0 - S$ where x_0 and S_0 are initial values of substrate and bio sorry biomass and substrate concentrations. So now you can relate x with S let us see how do we relate y by S multiplied by $S_0 - S + x_0$ should be giving us x . So I think this is pretty simple.

Now x we know is an exponential function of time is not it this is the exponential growth. So your $\ln x$ by x_0 is equals to μ into t now this μ here is nearly equal to μ_{max} . So now your batch time can be given as 1 by μ_{max} multiplied by $\ln x$ by x_0 is not it. Now here further the x can be substituted as the equation 1 here let us call this as equation 1 and this has equation 2. So if we do the substitution of x in terms of the entire portion which I have put in curly brackets in equation 2.

Then you can calculate the value of batch time because rest of everything in this equation is known we know S we know S_0 we know x_0 so everything is solvable. So this becomes your batch time this is what is given this is what was given here as well as this equation. So if you expand this you will reach where v were this is $x_0 + Y_{x/y} S_0 - S_f$ divided by S_f is your the final concentration which we were calling as S versus x_0 .

This is going to give you the batch time. So if you now put up all the known values then you will get an answer of 11.3 hours. Now once you know the batch time then you can calculate the final biomass produced by the equation given in the end which is the exponential growth rate how the biomass changes as a function of batch time. So at the end of the batch time you will substitute t in the form of batch type and the culture is growing at its μ_{max} .

So the initial biomass is known so you can calculate the final biomass concentration. So it comes out to be 16.5 grams per liter.

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Total mass of cells (X) produced during the batch culture

$$X = (X_f - X_0)V$$

$$= \underline{16400 \text{ kg}} \quad \text{--- ①}$$

Total time for the culture

$$t_T = t_b + t_d$$

$$= \underline{16.35 \text{ h}}$$

The number of batches carried out in one year

$$\text{No. of batches} = \frac{365 \text{ days} \times 24 \text{ h}}{16.35 \text{ h}}$$

$$= \underline{536 \text{ batches}}$$

Annual biomass production = X * No. of batches

$$\underline{\text{tons/year}} = 8790.4$$



Now the next part of the question it is asked how much total mass actually it is the same part. So we had to calculate the annual biomass produced using this process. So the total mass of cells which can be produced from this batch culture will be now you know the X f you knew X 0 and you know the batch volume. So now you can calculate the mass of cells produced after each batch. This turns out to be 16400 kgs.

Now the total time before we run the second batch will be the batch time plus the downtime now we know the batch time and we have also given in the equation we have been given in the problem the downtime. So we will add the batch time with the downtime and it turns out to be 16.3 hours as the total time. So the number of batches which can be carried out in one year will be 365 into 24 divided by the total time because your batch time is in hours.



So it turns out that the batch time the number of batches which can be run annually will be 536. So the annual biomass production can be now calculated after one batch we knew from equation one on this slide how much could we produce till we run the second batch. So now if you know the total number of batches which can be run and then you can multiply it with the amount of biomass which you will obtain after running one batch you will get the total amount of annual biomass produced which is 8790.4 tons per year.

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(ii) Increasing the annual biomass production to 10000 tons/year.

The volume of bioreactor will be 1137.6 m³

Handwritten notes: $1000 \text{ m}^3 \rightarrow 8790.4$ tons, $? \rightarrow 10,000$ tons

In the second part of the question it was asked what size of the reactor should be chosen if the demand of the biomass produced is raised to 10000 tons per year. Now to solve the second part the reactor volume given for the batch process was 1000 meter cube. Now if 1000 meter cube reactor is giving us from the first part 8790.4 tons per year. So 8790.4 tons then what volume will be needed for 10000 tons both are per year basis. So that is a straightforward problem and then the answer is 11037.6.



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Example

A strain of *Escherichia coli* has been genetically engineered to produce human protein. A batch culture is started by inoculating 12 g cells into a 100 L fermenter containing 10 g/L glucose. The maximum specific growth rate of the culture is 0.9/h (assuming that the culture is growing at maximum specific growth rate in the exponential phase); the biomass yield from glucose is 0.575 g/g.

Handwritten notes: $X_f = X_0 e^{\mu t}$, $S_f = 0$

(a) Estimate the time required to reach stationary phase.

Let us see another problem a strain of E-coli has been genetically engineered to produce human proteins okay a batch culture is started by inoculating 12 grams of cells into a 100 liter fermenter containing 10 grams per liter glucose. So what all is given to us they have given us the initial cells which have been inoculated the starter cells the volume of the fermenter. So

we can calculate the initial biomass concentration is not it the amount is given to us and it is 100 liters so it turns out to be 0.12 grams per liter containing 10 grams per liter of glucose.

So the substrate initial concentration is also given to us which is 10 grams per liter. The maximum specific growth rate of the culture is given as 0.9 hour inverse. So μ_{max} is also given to us which is 0.9 hour inverse. Now it is also said to assume that the culture is growing at its maximum specific growth rate in the exponential phase. So which means that your X is equals to $X_0 e^{\mu t}$ in the exponential phase.

So now here μ can be replaced as μ_{max} the biomass yield from the glucose is given as which means $Y_{x/S}$ which is given as 0.575 grams per gram of glucose consumed. They are asking us to calculate the time required to reach the stationary phase. So students if you remember when does a culture reaches the stationary phase? If you remember the batch growth curve it starts the exponential phase starts and it reaches the stationary phase once the substrate is depleted and this is our exponential phase.

And the culture is growing at its μ_{max} so we can assume it is a constant slope. So this is our μ_{max} and so now we need to calculate the time required to reach the stationary phase so during the exponential phase as I had written earlier it is a function of exponential function of time. So now X_f will be equal to $X_0 e^{\mu_{max} t}$, X_f stands for the final biomass concentration μ_{max} multiplied by the time what time to reach the end the maximum biomass?



This is what has been asked let us call it as the batch time t_b . So now how do we calculate the time? Any guess right, so if you remember it will reach the stationary phase when the substrate is consumed completely. So there is substrate depletion then only it will reach the stationary phase. So you can assume that the substrate the final substrate concentration is nearly zero.

Now $Y_{x/S}$ is given to us so now $Y_{x/S}$ and what all is given to us. How it relates the x and S the $X_f - X_0$ divided by $S_0 - S$, S_0 is given to us S is nearly 0, X_0 is given to us except we need to calculate $Y_{x/S}$ is also given to us. So now equation 1 can give us the value of the final biomass is not it. Now from the equation 1 if we calculate the final biomass concentration from equation 2 we can then calculate the batch time or the time required to reach the end of the log phase and then we'll be able to answer the part 1 or a.

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S_0, S_f, X_0, X_f, S_0
 X_f/S_0

Example



A strain of *Escherichia coli* has been genetically engineered to produce human protein. A batch culture is started by inoculating 12 g cells into a 100 L fermenter containing 10 g/L glucose. The maximum specific growth rate of the culture is 0.9/h (assuming that the culture is growing at maximum specific growth rate in the exponential phase); the biomass yield from glucose is 0.575 g/g.

(a) Estimate the time required to reach stationary phase.
(b) What will be the final cell density if the fermentation is stopped after only 70% of the substrate is consumed?



Now let us see part B. What will be the final cell density if the fermentation is stopped after only 70% of the substrate consumed. So generally for part a what did we assume it was said that till the end of the log phase the reactor has been run which means so that we could assume that the substrate has completely depleted. Now they are saying rather than assuming that what if the reactor is harvested when the final substrate concentration is 30% of the initial cups it concentration then only 70% of the substrate is consumed.

So now you know the initial substrate concentration and you know the desired conversion which is 70%. So now you can calculate the final substrate concentration so now what is given to us the initial substrate concentration is given the final substrate concentration is can be obtained after 70% of the desired conversion of the initial substrate $Y_x \text{ by } S$ is also given and we know how $Y_x \text{ by } S$ relates the biomass production with the substrate consumption $X_f - X_0$ divided by $S_0 - S_f$ is not it f stands for final O stands for the initial value.

Now if this is known this is known S_f is also known X_0 is also known we can easily calculate the final biomass now.

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(i) $X_0 = 12\text{g}/100\text{L} = 0.12\text{g/L}$
 Assume that stationary phase is reached when $S_f = 0$

$$t_b = \frac{1}{\mu_{\max}} \ln \left[1 + \frac{Y_{X/S}}{x_0} (s_0 - s_f) \right]$$


$t_b = 4.3\text{h}$


(ii) If only 70% of substrate is consumed, $S_f = 0.3 S_0 = 3\text{g/L}$
 substituting the S_f value in the above equation, gives

$t_b = 3.94\text{h}$

Substituting for t_b gives

$$X_f = X_0 e^{\mu_{\max} t_b} = 4.16\text{g/L}$$





Let us see the solution now for the first problem which we discussed X_0 we can calculate given the volume and the mass of cells which are added in the reactor so the concentration at time t is equals to 0 when the cells start dividing it is or reach start getting into the exponential phase can be assumed as is 0.12 grams per litre. Now we need to calculate the time when the culture reaches stationary phase so here we are saying that the final substrate concentration is zero at the start of the stationary phase.

The culture is growing at its maximum specific growth rate and from the earlier problem if you remember when we used the two equations X is equals to $X_0 e^{\mu t}$ and $Y_{X/S}$ is equals to $X - X_0$ divided by $S_0 - S$ and in the initial problem we did we used these two equation and here μ was again can be assumed as μ_{\max} then we could relate the batch time as a function of the substrate concentrations and the initial biomass because the final biomass is not known.

So there can be more than one ways to solve a problem so here you can calculate the batch time after putting up the known values in this equation. Now to solve the second part if only 70% of the substrate is consumed the final substrate concentration would be 0.3 times S_0 30% of the initial substrate concentration which turns out to be 3 grams per litre. Now if you substitute this S_f value in the previous equation here you can calculate the value of the time which will be required for 70% conversion of the substrate.

So once you know that time of batch for 70% conversion of the substrate you can then substitute in this equation and find the value of final biomass concentration obtained which is this value.