Bioreactors
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Lecture - 10
Batch growth kinetics

Welcome to lecture number 10, for the NPTEL online certification course on bioreactors, the 10-hour course. Today, we will a begin module 3 which is on analysis of common bioreactor operating modes. Let me maximizes the screen here.

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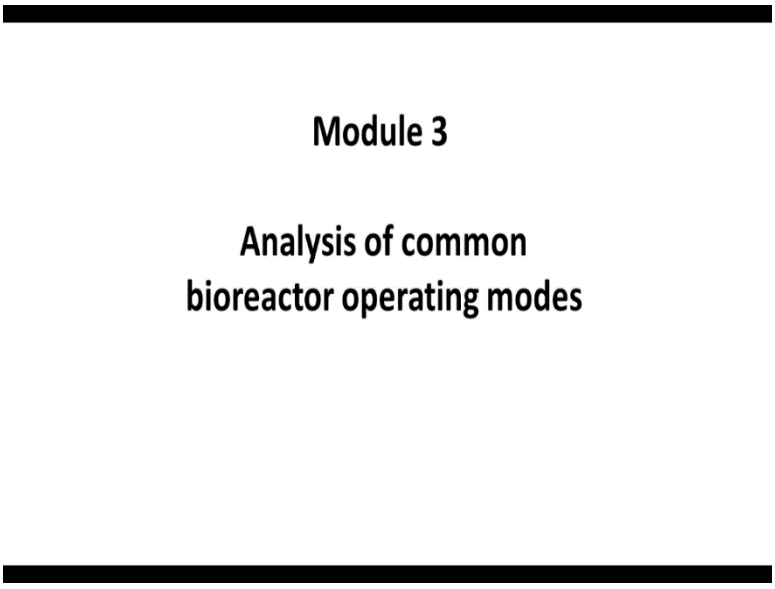

Analysis of common bioreactor operating modes.

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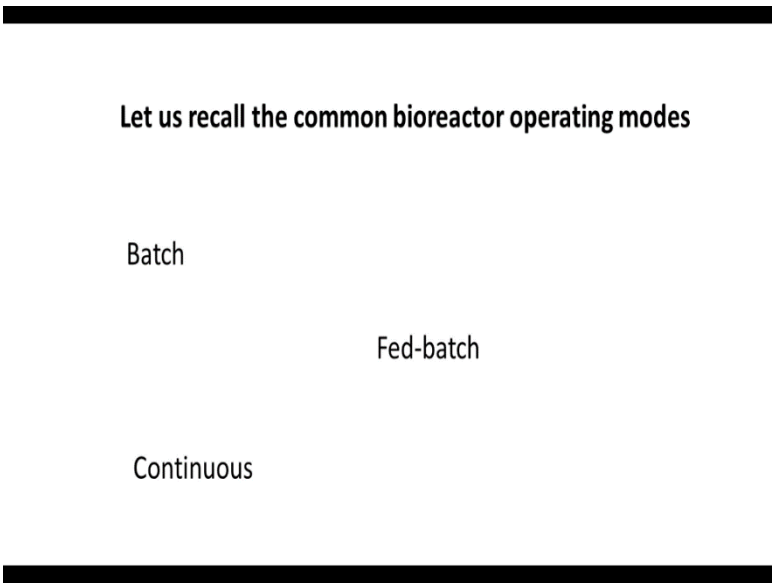

Let us first recall the common bioreactor operating modes that we saw in the previous module. We first saw batch as the operating mode. A batch is nothing but, you add everything in and then at start time everything is already there, this no addition or removal. The process takes place inside the batch reactor with no addition or removal and at the end of the batch time you take the contents out and go for processing. The batch time begins when everything, after everything has been added and the batch time ends before things are removed, that is the batch mode of operation.

We also saw the continuous mode of operation, where there is a continuous stream in and a continuous stream out of the bioreactor and the processes simultaneously take place when there is a continuous flow that is happening. In between the two, hence, the batch and the continuous we had the Fed-batch where you could start out as a batch and then you could have intermittent feeding or intermittent removal or even continuous feeding and continuous removal or both, you know as long as it is not both continuous in and continuous out simultaneously any combination is called, any other combination is called a fed-batch. These are the 3-common bioreactor operating modes the batch, the continuous and the fed-batch. Let us look at the analysis some basic analysis of these modes and such an analysis would help us answer design kind of a question. Questions such as, how long do we need to operate the bioreactor for, for a particular desired goal?

and so on. That would be a very basic design question that one would want to ask. And then, based on that there could be other complex design questions that one can answer through such analysis.

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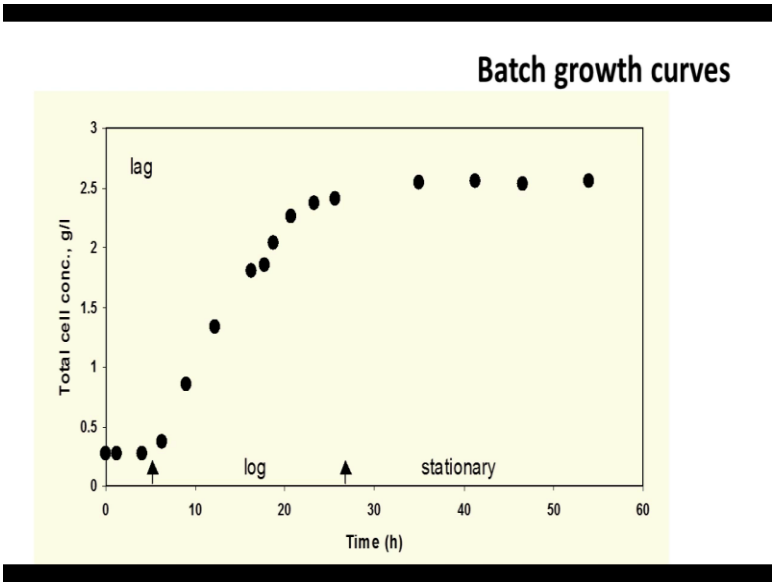

In this lecture, let us focus on the Batch bioreactor. The Batch bioreactor, we are first taking up because it is a very common mode of operation especially in the industries. It is very powerful that way, simple but powerful. It is easy to operate compared to the other modes and that is one of the reasons why it is preferred in the industry, the batch mode of operation. What is shown here, is some of our own data from our lab, a long time ago, this is total cell concentration. Remember, we talked about total cell concentration and viable cell concentration. This is total cell concentration which has been measured through what we called OD actually, cell scatter and then calibrated and we have used a calibration curve to convert OD to gram per liter. I think this is *Xanthomonas campestris* if I remember, right. I just took one representative batch growth. The total cell concentration in gram per liter is on the y axis, the time of growth is on the x axis. As we can see till about, let us say 5, 6 hours, there is no change in the total cell concentration. Around 6 hours, the cell concentration starts to increase till about 28 hours and then it goes flat. The phase in which, the initial phase in which there is no change in the cell concentration - total cell concentration is called the lag phase. The

region where the cell concentration changes rapidly is called the log phase, we'll look at the reason a little later. And then, it reaches what is called a stationary phase where there is no increase in cell concentration.

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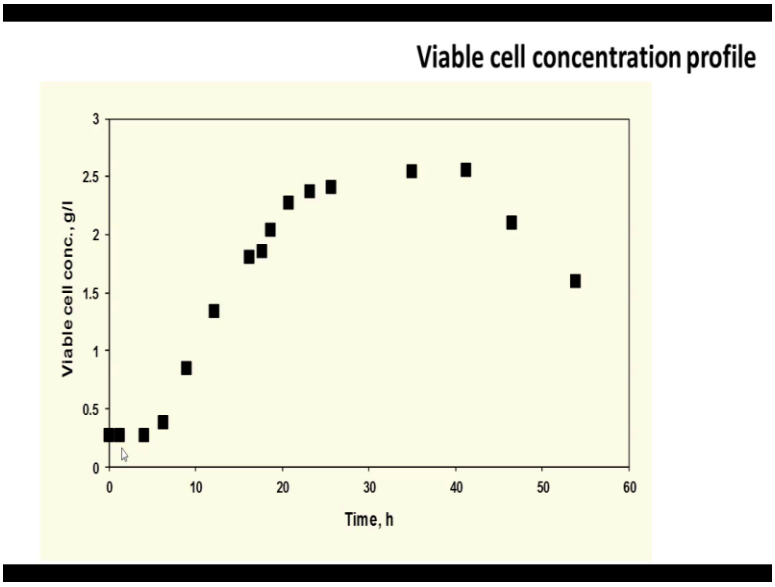

In slight contrast, let us look at the viable cell concentration profile for the same organism. The viable cell concentration gram per liter is given here, time in hours, this is a total cell concentration, the scale is pretty much the same 0 to 3, here again zero to three, but this is viable cell concentration. This was most likely obtained by the plating method, *Xanthomonas* plating method. As we can see, there is a good over lap of the viable cell concentration with the total cell concentration here right, in this region here till about this region and till about here, there is a good over lap. And then at around 40 hours, it starts going down as expected because the viable cell concentration would dropp here, after some time the stationary phase you enter the death phase which will show up in the viable cell concentration profile. It does not show up for a long time in the total cell concentration profile. That is actual data.

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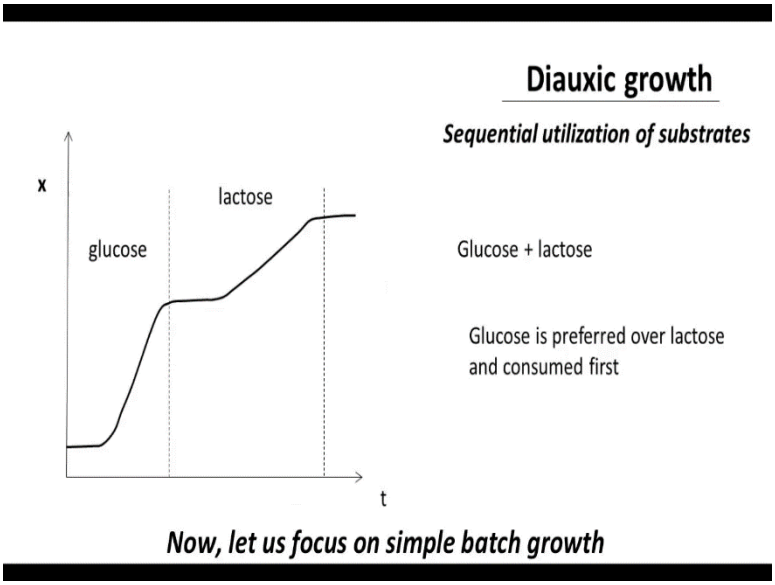

So, that is a simple batch where probably there was one limiting substrate, one main substrate and the cells grew on that substrate most likely glucose. There is another type of growth that is sometimes seen which is called the diauxic growth, sometimes they call it diauxy and so on so fourth. Which essentially arises, because of sequential utilization of substrates one after the other - sequential utilization.

For example, if there is a mixture of glucose and lactose. Let us say for a typical organisms such as *Eschericia coli*. Then glucose is preferred over lactose and consumed first. There is a well known mechanism for this to happen. In molecular terms it is known the lac operon, so on so fourth. We will not get into that; we understand very well why this happens in *E. coli*. However, for our purposes the growth curve would look like this. If you plot total cell concentration x versus time, there is an initial lag phase and then there is a log phase and then this seems like stationary phase, but it is a actually secondary lag phase and then growth occurs. And when you do the analysis of glucose and lactose, one finds that glucose gets consumed here first and then lactose gets consumed. As you can see the growth rate, which you would get out of this, which will we see how to get, would be higher here compared to the growth rate on lactose. The specific growth rate on glucose would be higher than the specific growth rate on lactose that is the usual way in which happens. The preferred substrate gets taken up first with a

typically higher growth rate, specific growth rate followed by the other substrate. This is also seen and it is good to know this, even in an introductory course.

Having said this for our analysis, let us focus on simple batch growth that we saw earlier. One organism, one substrate and so on so fourth, one limiting substrate.

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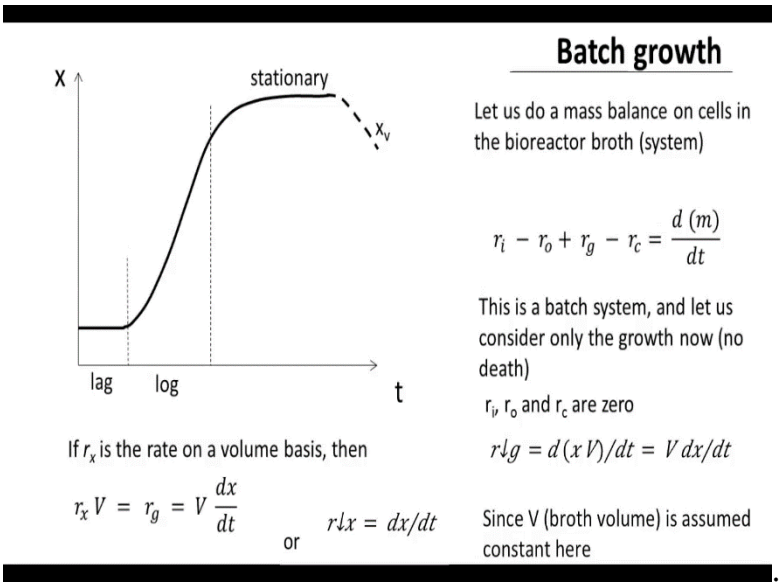

Batch growth, as we saw with real data. Cell concentration versus time, a lag phase, something called a log phase and a stationary phase. And if you plot viable cell concentration you would also see a death phase, where the viable cell concentration goes down. The solid line is the total cell concentration, the dotted line or the dashed line is the viable cell concentration.

Now, let us do a mass balance on cells in the bioreactor broth. The bioreactor broth - the liquid in the bioreactor is what we are going to take as our system, to do this mass balance on cells. This is our equation that we have seen right from module 1, these are mass rates. Mass rate of input minus mass rate of output plus rate of generation minus rate consumption all on a mass basis equals the rate of accumulation of mass. Since, we are doing the balance on cells this would be written for the mass of cells in the batch bioreactor.

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

Since, it is batch and let us consider only the growth, this phase for let us say only this phase for now, in other words there is no death that we are going to consider. There is no rate of input, there is no rate of output because it is a batch, that is the basic definition of a batch. And because there is no death, there is no consumption of cells and therefore, r i, r o and r c are 0, those 3 rates are zero. Therefore, r g which is the growth rate on a mass basis equals d m dt here.

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

If we replace m in terms of cell concentration because the cell concentrations are the ones that are easily measured. We would like to write things in terms of measured quantities because those are the ones that are measured and they can be used to make decisions. So, r g on a mass basis is d dt of m which can be written as x into V, cell concentration is mass of cells for unit volume times the volume of the system or the broth that would be m.

$$r_g = \frac{d(m)}{dt} = \frac{d(xV)}{dt}$$

And in the case of batch growth there is no change in the volume of batch right, there is no addition, there is no a depletion or taking away things from the batch and therefore, volume remains constant. If volume is a constant, it is not going to change with time and therefore, V can be taken out as a constant here, out of this derivative and therefore, it becomes V times d x dt.

$$r_g = \frac{d(xV)}{dt} = V\frac{dx}{dt}$$

Now, if r x is the rate on a volumetric basis, again those are the ones that are directly measured. So, we will write it in terms of them. Then r x into V would be equal to r g, you know this is the, on a concentration or a volumetric basis this is the rate, in other words the rate of change of cell concentration with time is what r x is. Therefore, rate of change, of cell concentration times the volume would be the rate of change of mass concentration sorry rate of change of mass not a concentration rate of change of mass. Therefore, r x times V equals r g and that equals V d x dt from here. So, if we equate this and this r x is nothing but, d x dt.

$$r_x V = r_g = V \frac{dx}{dt}$$

$$r_x = \frac{dx}{dt}$$

So, under the special case of the batch bioreactor, the volumetric rate equals the rate of accumulation of the cell concentration. This we have already seen as a special case of balance. And we also said that this is the way you would have been introduced in your school and that is rather limited to the batch case and do not take it forward. This aspect will become clear when we discuss continuous processes. The concept of a rate is a lot more general where as the rate being equal to an accumulation rate is valid only for a batch system.

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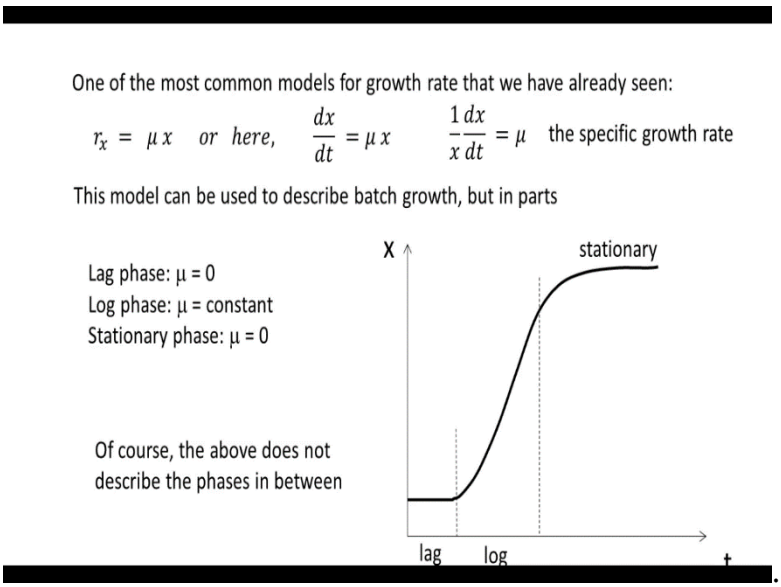

One of the common models for growth rate that we have already seen; in fact the first model that we saw was r x, you know this is on a volumetric basis r x equals mu x or in this case d x dt.

$$r_x = \mu x \quad or \quad here, \quad \frac{dx}{dt} = \mu x$$

The accumulation rate equals mu x we are replacing d x, sorry we are replacing r x with d x dt, d x dt equals mu x. In fact, mu can be defined in such a situation or here as 1 by x, d x dt even it becomes easier to see the meaning of the term, that is the reason I have given it here, it is still, the meaning of the term is still valid. It is just easier to see it here 1 by x, d x dt. It has been normalized with respect to cell concentration and therefore, it is called the specific growth rate.

$$\frac{1}{x}\frac{dx}{dt} = \mu$$

This model can be used to describe batch growth, but in parts. These are the various parts of the batch growth that we have seen, the variation of cell concentration with time the lag, the log and the stationary phase.

In the lag phase, mu is 0 you know d x dt is 0, there is no change in the cell concentration. In the log phase which is here, which is probably what you have been exposed to so far, mu happens to be a constant. And in the stationary phase, again there is no change in cell concentration therefore, mu is 0.

There are some phases in between the late log phase, the early log phase or the early stationary phase as it is called here. These phases are not very clearly described by this equation, but it may not be very necessary to know them or to describe them for our ends to make a design decisions and therefore, we will not get into that. There are ways of doing them, there are ways of including them in the mathematical representation, we will not look into those aspects in this particular course.

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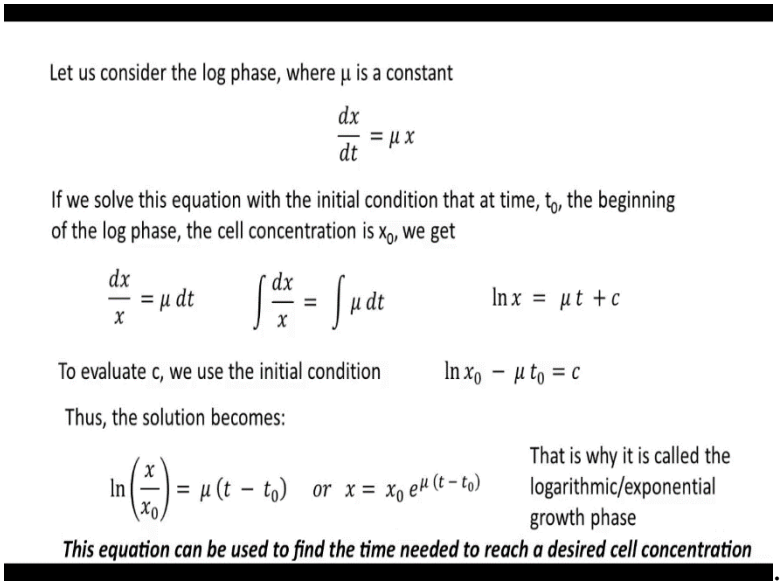

Now, let us consider the log phase alone. That is where we are going to focus on. The reason will become clearer as we go along. In the log phase, mu is a constant. Therefore, dx dt equals mu x, mu can be taken to be a constant here.

$$\frac{dx}{dt} = \mu x$$

If we solve this straight forward first order differential equation, you know the first order differential equation you need at least 1 initial condition to solve. So, the initial condition is as follows at time t equals t 0 which is the beginning of the log phase, its not the beginning of the batch, it is the beginning of the log phase, the cell concentration is x zero. x zero may not be very different from the concentration at the start of the batch but, sometimes there might be a difference. It has not changed much, but it has changed a little bit. So, you need to keep, you need to have clarity on what x zero actually is because this model where mu is a constant is applicable only to the log phase not to any other phase.

So, if we go about solving this equation, just a few steps, I have written down the steps here.

$$\frac{dx}{x} = \mu\, dt$$

Integrate both sides we get the integral of dx by x as ln x and here it is straight forward mu t plus c in definite integral.

$$\int \frac{dx}{x} = \int \mu\, dt$$

$$\ln x = \mu t + c$$

We have the initial condition here which can be used to evaluate c if you put in the initial condition at time t equals t zero, x equals x zero. Therefore, ln of x equals mu t zero plus c or c equals ln of x zero minus mu into t zero, this is our c.

$$\ln x_0 - \mu t_0 = c$$

So, if you substitute it back into the expression here, the solution becomes:

$$\ln x = \mu t + \ln x_0 - \mu t_0$$

If we combine similar terms on either side, then ln of x minus ln of x naught, which turns out to be:

$$\ln\left(\frac{x}{x_0}\right) = \mu (t - t_0)$$

And therefore, x equals:

$$x = x_0 e^{\mu (t - t_0)}$$

This is the description of the variation in cell concentration x with time. Since, this is exponential and this is a log we call the phase as either an logarithmic phase which corresponds to this or an exponential growth phase which actually describes the variation of cell concentration with time. That is actually why it is called the log phase. This equation can be used to find the time needed to reach a desired cell concentration. That is a straight forward design application.

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Thus, if the parameters μ, t_0 (lag phase time), and x_0 are known, we can predict the total time from the start of the batch, t, to reach x:

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

Practice problem 3.1.

In a batch bioreactor, the concentration after inoculation was 0.5 g l^{-1}. The lag phase usually lasts 20 min under these conditions. Assuming that the cell concentration at the start of log phase was not significantly different from that immediately after inoculation,
(a) estimate the time needed for it to reach 4 g l^{-1}. The specific growth rate for this organism under these conditions is 0.5 h^{-1}.
(b) what is the time needed for the cell concentration to double in the log phase?

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If the parameters mu t naught which is the lag phase time and x naught which is the concentration at the beginning of the log phase which may not be very different from the initial cell concentration, we can predict the total time from the start of the batch t to reach x as this. It is 1 by mu ln of x by x naught, this is what we get from our previous thing here. 1 by mu ln of x by x naught, if t naught can be taken as a zero plus our t naught which is the time that the batch spends in the lag phase. So, lag time it is here this is the time for logarithmic growth starting from x zero to reach x this would be the total time of the batch, if we are interested in reaching a certain cell concentration x beginning with the cell concentration of about x zero. I am assuming that there is no change in cell concentration in the lag phase.

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

Having said this, let me assign this problem here we. Will continue a little bit more. We will continue and do a little bit more in this lecture itself for completeness, but I would like you to work this out at the end of this lecture to get some practice in the batch

bioreactor analysis. The problem reads in a batch bioreactor the concentration of the inoculum was 0.5 gram per liter, this is the initial cell concentration. The lag phase usually lasts 20 minutes under these conditions. Assuming that the cell concentration at the start of the log phase was not significantly different from that immediately after inoculation, (a)estimate the time needed for it to reach 4 gram per liter. The specific growth rate for this organism under these conditions is 0.5 hour inverse. (b)what is the time needed for the cell concentration to double in the log phase? This is the problem. Please work it out. It is an application of the earlier derived equation, we will of course see the solution in the next lecture.

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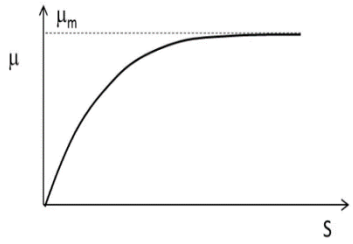
The above did not consider the effect of substrate on the specific growth rate, μ. We tacitly assumed that enough substrate was present, so that $\mu = \mu_m$. This need not always be the case. We have seen that



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

Monod model

Let us continue now, the initial analysis, what we have done so far, did not consider the effect of substrate on the specific growth rate mu. We had kind of tacitly assumed that enough substrate was present, so that mu equals mu m, you know mu versus S, mu changes when the S changes in the initial stages and then reaches constant value or an asymptotic value mu m, when the substrate concentration is above a certain level. We kind of assumed that we always had enough substrate so that, we were always at mu m for the specific growth rate. This need not always be the case and we have seen this. This is the mathematical representation of the variation, the monod model of mu's variation with S, the specific growth rate is a mu m times s by K s plus S.

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

This variation would effect the growth rate and therefore, that will ultimately effect the time that it takes to achieve a certain cell concentration in batch, if it is possible.

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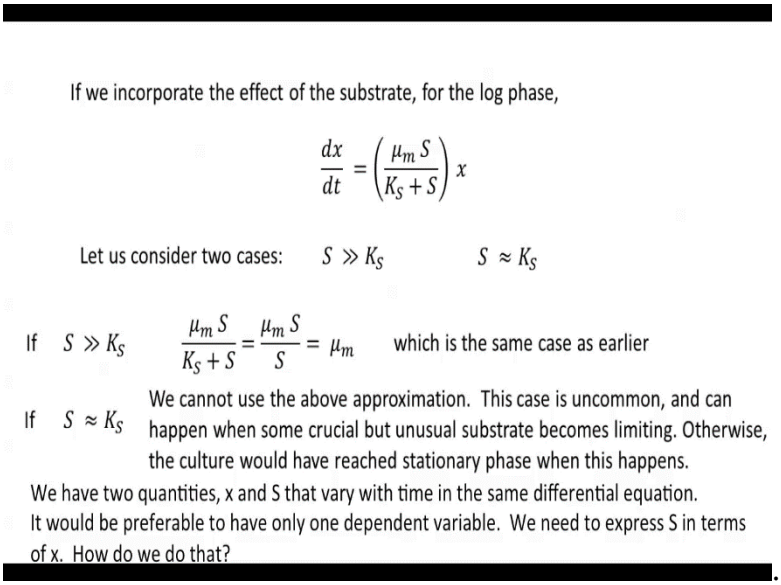

If we incorporate the effect of the substrate, for the log phase, the mu needs to be replaced by mu m S by K s plus S. So, dx dt equals mu x is still valid. Only the mu has been replaced with the substrate effect, to include the substrate effect mu m S by excuse me K s plus S.

$$\frac{dx}{dt} = \left(\frac{\mu_m S}{K_S + S}\right) x$$

Now, let us consider two cases for ease of analysis. The first case, is that the substrate concentration is much, much greater than the K s value.

$$S \gg K_S$$

Recall this K s is the substrate concentration at which we got half maximal growth rate. What we are saying is that we are somewhere here were the substrate concentration is much higher than K s, that is the condition that we are looking at first. If that is the case, then S is approximately equal to K s. I will tell you how this happens, mathematically. K s and S, they are added in the denominator. If s is much, much greater than K s let us say, just to illustrate that, K s is 1 and S is 1000, whether it is 1000 in the denominator or whether it is 1001 in the denominator, it is not going to make of a much difference to the value of this mu. So, you can very safely replace K s plus s with just s alone, if s happens to be much, much greater than K s. If we replace the denominator by s you have mu m s by S, S S can be canceled and you will be left with mu m alone and that is what is shown here.

$$If\ \ S\ \gg K_S, then\ \frac{\mu_m S}{K_S + S} = \frac{\mu_m S}{S} = \ \mu_m$$

Which is exactly the same case as earlier, only thing is that instead of saying dx dt equals to mu x we could directly say dx dt equals mu m x, we had actually tacitly assumed so earlier even in our earlier analysis, but here we can very confidently say that this is mu m times x, it is the maximum specific growth rate. Now is the second condition, if s is approximately equal to K s, then we cannot use the above approximation.

If $S\ \approx K_S$

You cannot say that you can replace K s plus S with S alone because both are comparable. This case is rather uncommon, in the case of a batch bioreactor and can happen when some crucial but unusual substrate becomes limiting. Otherwise, the culture would have reached stationary phase when this actually happens. That is you in the batch you typically make sure that you have enough substrate, but there are situations when this can arise and this also has great relevance in the continuous bioreactor analysis.

So, let us start looking at it here, where it has some application and then picking it up for the continuous case becomes a little easier also. We have two quantities here x and s that

vary with time in the differential equation and of course, you know that it is preferable to have only one dependent variable, either x or s not both, that becomes difficult here. So, if you can convert one in terms of the other s in terms of x preferably because we are looking at x, we have x all over, if we can express s in terms of x then, we can solve this equation. How do we do that? Some thoughts on that, something related to some concepts that we have seen earlier in the course in module 2.

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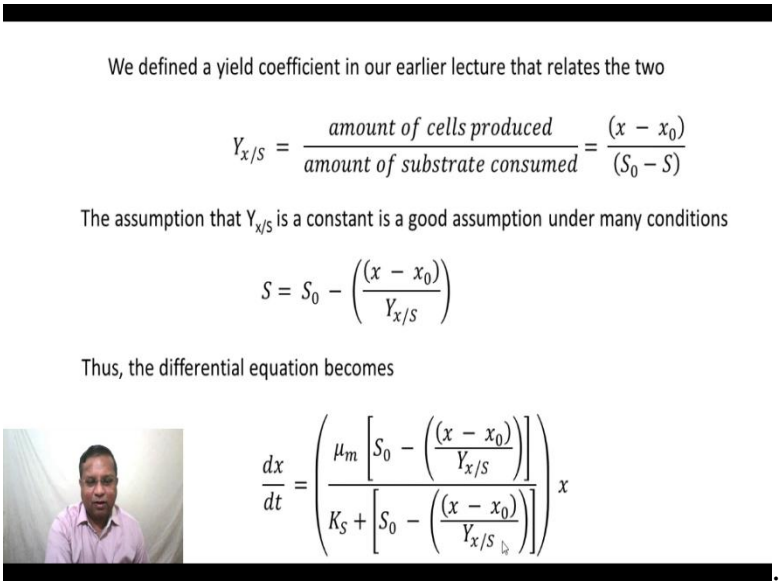

Yes, we could use what we called, what we defined as a yield coefficient in the earlier lecture or in the earlier module, that relates the cell concentration and the substrate concentration.

We know that Y x/ s, yield of cells with respect to substrate is nothing but the amount of cells produced by the amount of substrate consumed, in terms of the values that we have defined here. x minus x naught is the amount of cells, the concentration of cells produced by the concentration of the substrate consumed.

$$Y_{\frac{x}{S}} = \frac{amount\ of\ cells\ produced}{amount\ of\ substrate\ consumed} = \frac{(x - x_0)}{(S_0 - S)}$$

And the concentration is amount by unit volume, since we are dividing one by the other and the volume happens to be the same, you can cancel out the volumes. So, the amount of cells produced by the amount of substrate consumed can be replaced on both the numerator and the denominator by the concentrations and we would have the same yield coefficient. That is what we have done here. And the assumption that Y x/s is a constant is a good assumption under many different conditions.

If that is the case, we can use the yield coefficient to write s in terms of x, S_0 is a constant, S_0 is the total substrate concentration that is usually known. Similarly, x_0 is the initial cell concentration assuming there's not much of a change in the lag phase that is also known. So, we are actually interested in the variables x and relating the variable s and x. So, s equals:

$$S = S_0 - \left(\frac{(x - x_0)}{Y_{x/S}}\right)$$

Therefore, after substituting for S, the differential equation becomes

$$\frac{dx}{dt} = \left(\frac{\mu_m \left[S_0 - \left(\frac{(x - x_0)}{Y_{x/S}}\right)\right]}{K_S + \left[S_0 - \left(\frac{(x - x_0)}{Y_{x/S}}\right)\right]}\right) x$$

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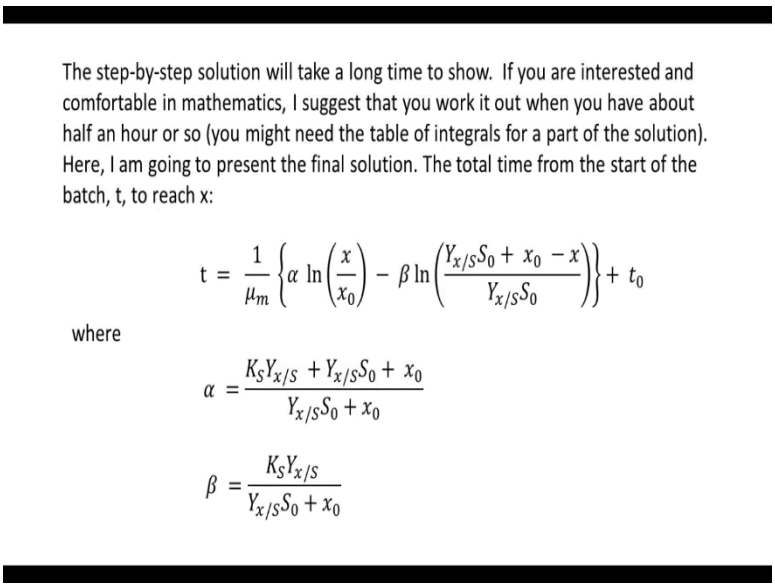

The step-by-step solution will take a very long time to show. Especially in the context of this course this is a total of 10 hours, this is probably going to take 45 minutes to show, all the algebra. So, if you are interested and comfortable in mathematics, I would suggest that you work it out, when you have about a half an hour or so. If you are good in math, it would not take more than that. You might also need a table of integrals for the part of the solution you do not want go and derive the well-known results. So, you need to use some well-known results also from the table of integrals. Here, I am just going to present the final solution we need to take it on face value.

If you are doubtful you go head and solve it and convince yourself, that it is indeed the solution. The total time for the start of the batch from the start of the batch to reach a certain cell concentration x is given as:

$$t = \frac{1}{\mu_m}\left\{\alpha \ln\left(\frac{x}{x_0}\right) - \beta \ln\left(\frac{Y_{x/S}S_0 + x_0 - x}{Y_{x/S}S_0}\right)\right\} + t_0$$

where

$$\alpha = \frac{K_S Y_{\frac{x}{S}} + Y_{\frac{x}{S}}S_0 + x_0}{Y_{\frac{x}{S}}S_0 + x_0}$$

$$\beta = \frac{K_S Y_{x/S}}{Y_{x/S} S_0 + x_0}$$

It will take quite a bit of algebra to show this, if you are good at math, interested please go ahead and show it to a convince yourself, that it is the indeed the case. I think it is a good time to stop here for this lecture. When we meet next, I will solve the problem that has been assigned. But please make all attempts to solve it, you need to pickup the skills of close ended problem solving at least. And if you follow the method some time soon, you would be able to solve complex problems.