**Biology for Engineers and other Non-Biologists**
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**Lecture Number 15**
# Culture Growth

Welcome to this lecture on 'Culture Growth'. It is popularly referred to as 'cell growth' and it actually represents the growth of a population of cells or a culture. So these terms will be used interchangeably; cell growth is a very 'common term', rather misleading because we are not looking at the growth of a single cell here. You already know from the previous lectures, that the cell goes through a cell cycle, during which its size changes, its volume and other things change as it goes through the cell cycle; and therefore you can probably look at it as some sort of a growth, from our perspective. But that is not what we are going to talk about in this particular lecture.

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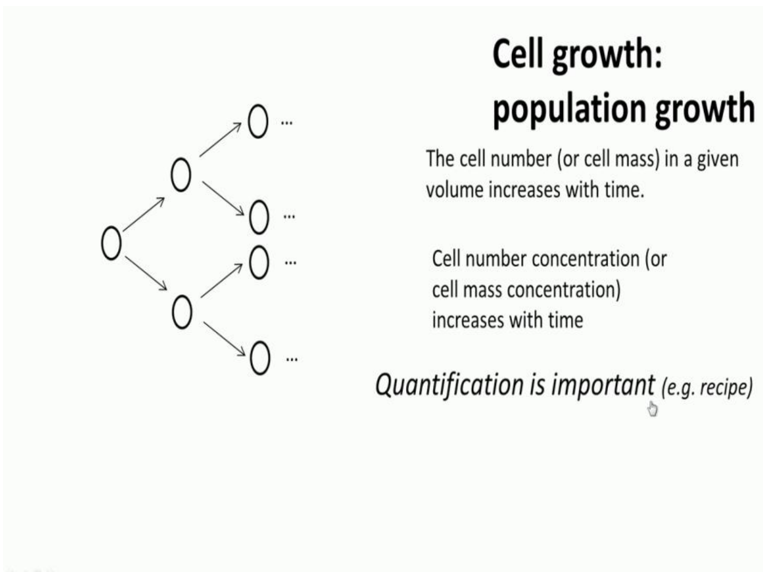


It is referred to as cell growth, but actually means the growth of a population of cells or the growth of culture. What we mean is actually this, a single cell divides to become two cells, it is either mitosis, of all the somatic cells, which yields two daughter cells or the meiosis, of germ cells, which yields two gametes. We, let us limit ourselves to somatic cells to understand the population growth here, and that is typically where it is used, predominantly in the production, from a production perspective.

And therefore let us look at mitosis of one cell giving two cells, and each one of those giving two cells and so on and so forth. It is this process that we are interested in in this particular lecture. The cell number, or the cell mass in a given volume, right, that is, what we are normally interested in, the cell number, number of cells in the given volume, or conversely, the cell mass or alternatively, the cell mass in a given volume increases in time because of this multiplication process. In other words, the cell number concentration or the cell mass concentration increases with time. When you normalize it with respect to volume, we call it concentration usually.

Cell number concentration or cell mass concentration increases with time and to know how it increases in a precise fashion is important. In other words, quantification is important; quantification is important in biology also. More and more, that is being realized more and more even by classical biologists nowadays.

I will give you a very simple example to understand why quantification is important. Suppose you are making a dish, cooking a dish, okay? There is always a recipe that we average people follow, we are not, let us assume that we are not great chefs who have an, an inherent feel for what they cook, and therefore they do not need all these things. That is inside them.

Let us say that we are average people, we are following a recipe to cook a dish. The, if the recipe says you add rice, you add milk, you add sugar, and so on and so forth, we will be completely lost, right? We need to know, okay let us make it a little more specific. Let us say that we are making cup of tea. If it says boil water, how much water do you boil? If it says add tea leaves or tea dust, how much tea dust do you add? Do you add an entire box, to make one cup of tea? All these are very common questions that come about even while making tea, right?

You need to know that you take, let us say, a cup of water, then you add, let us say, a teaspoon of tea powder, and if you are adding any masala to it, may be about a quarter teaspoon of it and so on and so forth. Therefore every single thing needs to have a certain measure associated with it for us to successfully make a dish, okay? So the quantification, one glass of water, one teaspoon of tea leaves and quarter teaspoon of chai masala and so on and so forth are very essential aspects to get the dish right. And therefore, quantification is very important in many different things, especially if you want to do things reproducibly.

In biology too, this aspect is gaining more and more importance, and here, let us see how to quantify this population growth process, cell growth. For dynamic systems, dynamic means, which change with time. Biological systems are dynamic systems, including the cell, they change with time all the all the time.

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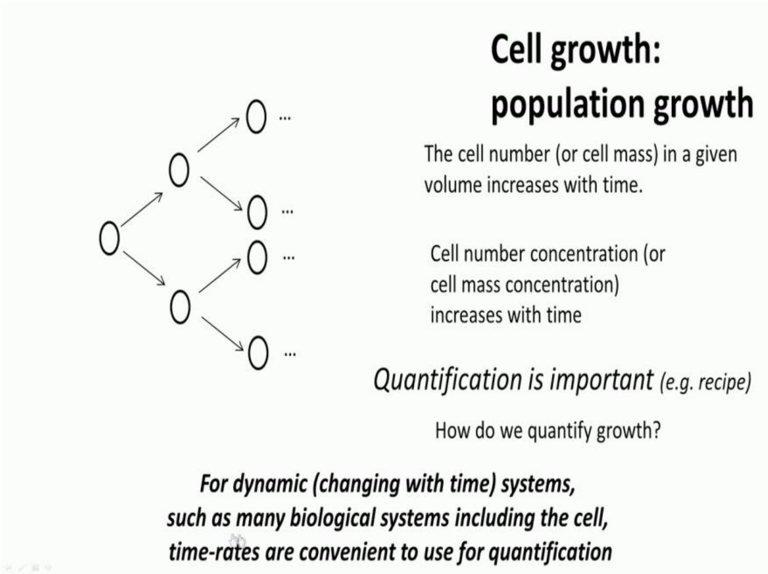


They go through life, right? So they, they are subject to changes at every single point in time. The time rates are convenient to use for quantification, this is something that has not been realized much, and I think it is best to present it during a first course in biology; that the time rates are rather important (becau) I mean are very important, it is it does not make much sense to deal with just masses in volumes and so on and so forth, when we are trying to quantify a dynamic system, such as a cell.

And the rate, time rates would provide us with easy answers to aspects that we are usually interested in, especially when we are trying to use biological systems for various different ends, as well as understand biological systems quantitatively so that it can be reproducibly used later on. To understand this, let me start at the very basics, and, so that we are all at the same level, everybody understands the need and everybody takes a certain view to that. Let us say that we are filling a water tank; water tanks are quite common in Chennai in summer, they provide water for use when water scarcity sets in. The typical volume, or let me say an average, kind of an average volume is about twelve thousand litres. These were kind of old tankers, twelve thousand litres. Nowadays you have eight thousand litres or sixteen thousand litres, depending on the size; twelve thousand is somewhere in the middle.

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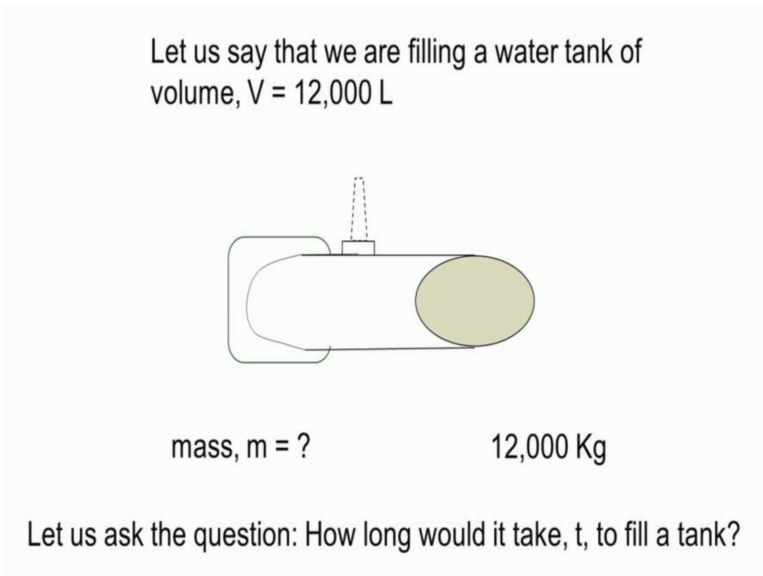


So I have chosen this for our representation. The volume here is twelve thousand litres. So what is the mass? You know that the density of water is one gram per centimeter cubed, one one gram per ml, or ten power three kilogram per meter cubed, okay? So if you are dealing with twelve thousand litres, that is twelve meter cubed, right, and therefore, the mass is volume into density, which would be twelve thousand kilograms. This is rather straightforward for people who have some background in physics, maths and so on.

Now let us ask the question, how long would it take to fill the tank, and let us call that time t. How long would it take t to fill this tank?

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| $r_{in}$, Input rate (Kg $s^{-1}$) | t, time (s) |
|---|---|
| 10 | 1200 (20 min) |
| 20 | 600 (10 min) |
| 50 | 240 (4 min) |

If we know the ***rate*** of water input, $r_{in}$, $t = m/ r_{in}$

This can be easily answered if we know the input rate of water into the tank. If the input rate happens to be ten kilogram per second, the time taken would be thousand two hundred seconds, or twenty minutes. If the input rate is twenty kilogram per second, the time taken would be six hundred seconds or ten minutes. If the input rate is fifty kilogram per second, the input, the time would be two fourty seconds to fill he tank, or four minutes, okay?

The, in other words, if we know the rate of water input, okay, then the time is nothing but the mass, total mass divided by the rate. It becomes very straight forward. For further discussion, let us choose this time or this rate, twenty kilogram per second; ten minutes is the usual time that it takes to fill tankers here. So that is what we are going to choose for further discussion.

Remember, input rate is twenty kilogram per second. Now, let us complicate things slightly. The first one is straight forward, it did not really need a certain different view to answer, only thing is that if you had known that view, the rate view, it was a very straight forward answer. Even otherwise you would have got into that answer by some roundabout means.

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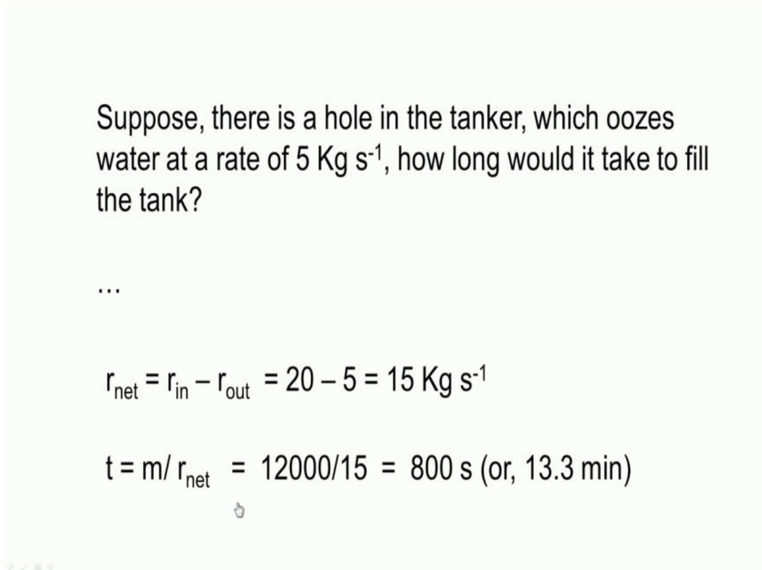


Now let us complicate the question that we are asking or the situation that we are in. Suppose, there is a hole in the tanker, which oozes water at the rate of five kilogram per second. Now, how long would it take to fill the tank, right? If we need to answer such questions, if we start looking at mass volume, there is going to be total confusion, okay you can try that. Many people do this; even engineers who have gone through courses in material balances do not internalize it, do to internalize the concept of rate and kind of intuitively get into mass and volume and so on so forth, and get confused.

Whereas, if you know the net rate, that is the rate of input minus the rate of output, water is coming in at twenty kilogram per second, going out at five kilogram per second; so the net rate is fifteen kilogram per second, and it is a one step answer to find the time, it is mass by the net rate. Twelve thousand kgs by fifteen kilogram per second, that is eight hundred seconds or thirteen point three minutes; divide by sixty, thirteen point three minutes. So if you know the rate, if you focus on the rate, it is a one step answer to the kind of information that we engineers or quantifiers are interested in. Biology is no different.

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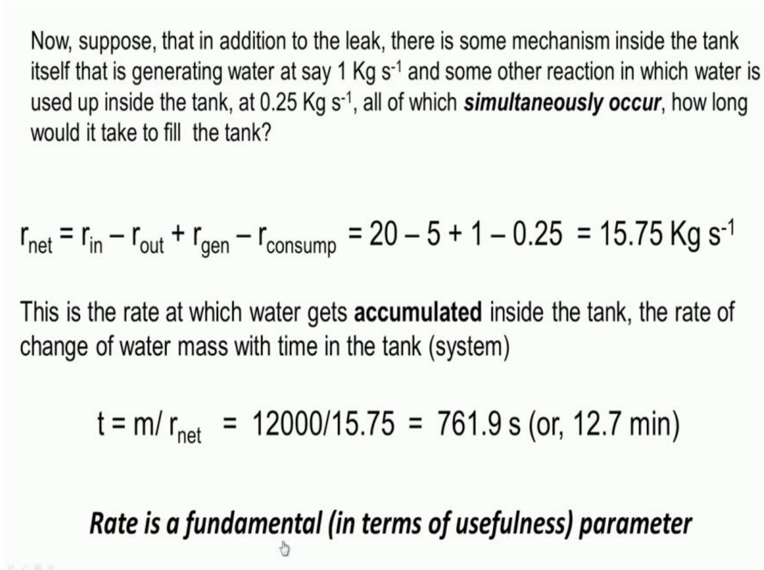


Let us complicate things even further. Now suppose that in addition to the leak, there is some mechanism inside the tank itself that is generating water at one kilogram per second, okay? Fictitious, but let us consider this. Some reaction inside the water, inside the tank that is generating water, and some other reaction in which water is used up inside the tank at a rate of point two five kilogram per second, and all of them simultaneously occur, okay, which is usually the killer for any intuitive kind of an approach with mass and volume, okay? All these processes simultaneously occur, there is filling in, there is water oozing out through the hole, there is water being generated and there is water being consumed. Okay?

And all of these are simultaneously occurring. If this is the case, how long would it take to fill the tank? And this becomes very straight forward, I mean if you take the mass and if you keep thinking about the mass and volume and so on so forth; you are welcome to do it, but most likely you will probably never arrive at the answer. Whereas, if you take the rate route, the net rate is nothing but the rate of input minus the rate of output, plus the rate of generation minus the rate of consumption, okay? That happens to be fifteen point seven five kilogram per second, twenty is input, five is the output, one is the generation and point two five is the consumption; all rates that comes out to be fifteen point seven five kilogram per second as the net rate.

Now, if this is the net rate, this is the rate at which water should get accumulated inside the tank, right? Or in other words, the rate of change of water mass with time inside the tank, and we are going to call the tank as our system. Some of you would be familiar with the stem system, something on which we focus our attention, and we we are considering the the tank as our system. That is the case, it is a straightforward calculation one step, total mass is mass that it can hold is twelve thousand, the rate, the net rate at which it is getting filled is fifteen point seven five, and therefore the time to take is twelve thousand by fifteen point seven five, seven sixty one point nine seconds, divide by sixty, twelve point seven minutes. Okay?

So, rate is a fundamental parameter in terms of usefulness. In most engineering calculations, and that is something that needs to be internalized. This is true for all dynamic systems, the cell biological systems or all dynamic systems. So, we need to look at rates of things happening, their rates of processes happening there, for us to be able to meaningfully quantify anything to do with this cell, okay, this I would like all of you to internalize because this is pretty much the key for any meaningful analysis. I see a lot of people even after lot of experience have not internalized this particular concept.

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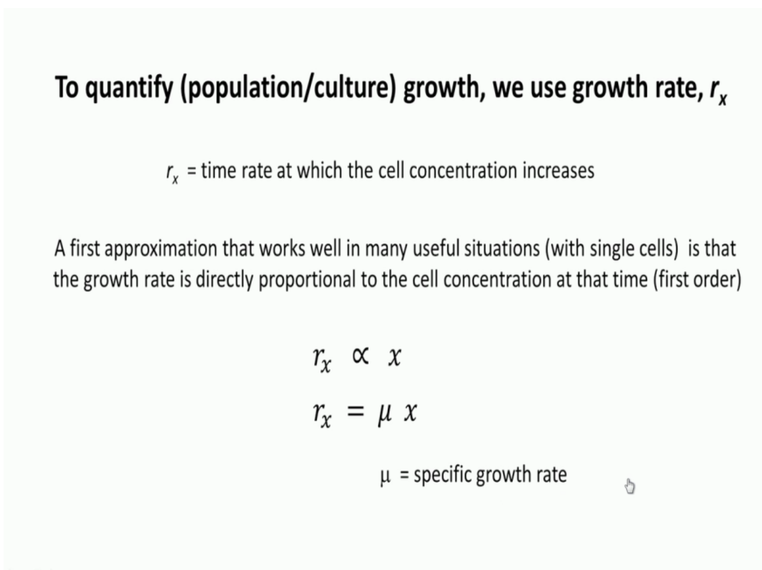


Now to quantify the population or culture growth, we are going to use something called a growth rate, which is represented as r subscript x, rx. The rx is nothing but the time rate at which the cell concentration increases, okay? Cell, see cell (con), talking of cell concentration makes sense, okay, because whether you have a one litre system, or whether you have a ten thousand litre system, this concentration can easily be considered the same or can be scaled up, can be considered to be equal in between these two systems. If you are looking at total cells in the one litre, it will be something; in the ten thousand litre, it will be ten thousand times that, okay?

So it is not kind of scalable. That is the reason why we normalize the cell number or the cell mass with respect to volume, we call that cell concentration, that remains the same across scales. It is scalable. For certain purposes, even cell concentration becomes a problem, we will, we will look at it if we have a need. Our first approximation that works well in many useful situations, especially when you have single cells, is that the growth is directly proportional to the cell concentration at that time. Okay? The, in other words, the rate of growth, growth rate is directly proportional to the cell concentration at that time, and this is nothing but the standard first order representation.

Rate is proportional to the concentration, okay, to the broth. And, the constant of proportionality, let us call it as mu, it is usually represented as mu, where mu is called the 'specific growth rate', okay?

It is  all  it is growth rate with respect to cell concentration 'rx by x', if you want to look at it that way; therefore that has been normalized with respect to cell concentration; therefore it becomes specific, and (there) therefore, it is called specific growth rate. Let me tell you another important principle that will be useful for analysis, I will just mention it to you and then leave it there, may be we will use one aspect of it.

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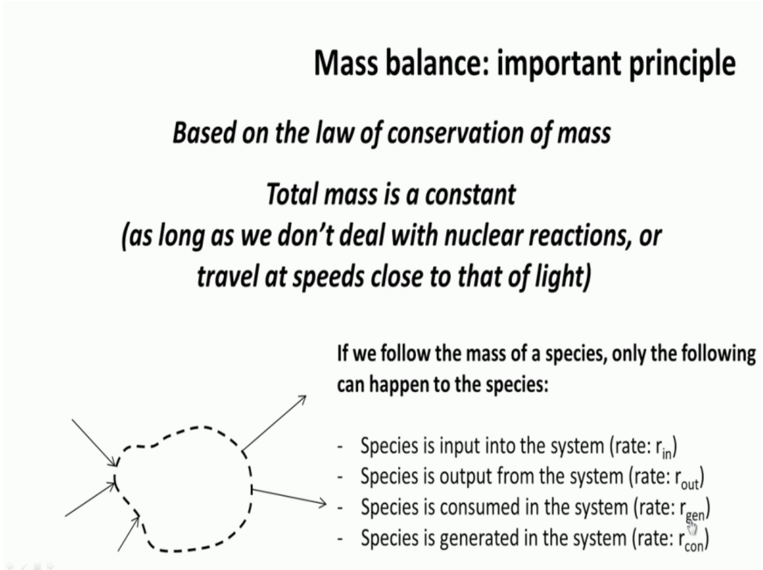


This is a very general principle. This is a principle of mass balance or material balance. As you would have guessed this based on the law of conservation of mass, which essentially says that the total mass is a constant as or the mass of a species is a constant as long as we do not deal with nuclear reactions or travel at speeds close to that of light. Right? I mean total species in in a certain formulation as a constant, we look at total mass is a constant as long as we do not deal with nuclear reactions where there is mass to energy conversion, or if we do not travel at speeds close to that of light, there is mass dilation and so on. Hence we will not consider.

As long as we are not dealing with these things; we are quite comfortable not dealing with these things for our purposes, then, if we focus our attention on something which is represented by these dotted these dashes, this could be anything, this could be a bioreactor (())(17:47), this could be the entire building, this could be an entire city and so on and so forth. All it says is that we are going to concentrate on that particular volume. There are inputs to this, there are output streams from this, and therefore, if we follow the mass of a species; we are looking at a species here, not total mass here; mass of a species, only the following can happen to the species.

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$$net\ rate = r_{in} - r_{out} + r_{gen} - r_{con}$$

$$net\ rate = \text{rate at which the species mass gets accumulated in the system, } \frac{dm}{dt}$$

If the species is cells ($x$)

$$r_{in} - r_{out} + r_{gen} - r_{con} = \frac{d\,(m_x)}{dt}$$

You can think about it, all all you want, if you can come up with something else, please let me know, you would be a genius or (whate), I mean, see whether you can come up with something else. This species is input into the system, may be at a rate of rin; the species is output from the system at the rate of rout; the species is consumed in the system at the rate of r, I am sorry, this is, this must be r (cons)(consum) consumed, con, and species is generated in the system at the rate of rgen, okay? This , these have been interchanged, please write this as consumed con, and this is generated.

Therefore the net rate is rate of input minus rate of output plus the rate of generation minus the rate of consumption of that particular species. The net rate is the rate at which the species mass gets accumulated in the system, and let us represent it by by ddt of m, okay? This is an accumulated mass; and if the species happens to be the cells x, then rate of input of cells minus rate of output of cells mass in terms of mass, plus the rate of generation of cells, minus the rate of consumption of cells equals the rate of accumulation of the cell mass. Remember, we are dealing with mass rates in all these cases, not concentrations.

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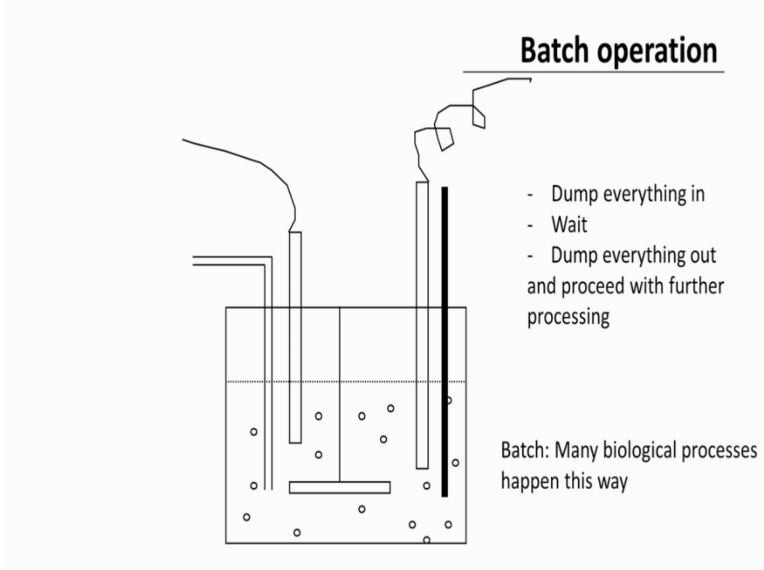


The batch operations are very common in the industry. A batch operation just means that, this is the representation of a bioreactor, you have a vessel, you have a highly controlled vessel, specialized vessel, you have a broth here with cells, you have a stirrer to keep the cells in suspension, and for other means, you have various probes here, probably the hbo probe, temperature probe and may be an air inlet here to provide oxygen to the cells. This is a, this is a typical stirred reactor, stirred bioreactor. The batch operation of the bioreactor is something like this.

We dump everything in, okay? Medium cells and so on and so forth, and at time zero, the operation starts after we have dumped everything here. Then, wait for the process to go to completion, then dump everything out and proceed with further processing. In such an operation, rather such an operation is called a batch operation. There are other kinds of operation, we will not get into that. And, very many biological processes happen this way, specially in biological and pharmaceutical industries, okay? The workhorse of a biological or a pharmaceutical industry is still a batch operation, because it is rather easy compared to the other processes to carry out, given the various constraints.

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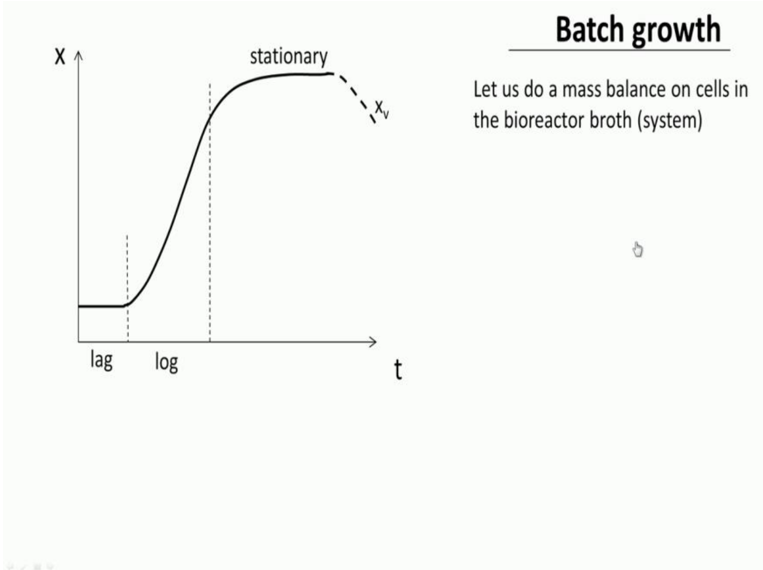


So, we are going to consider batch growth, growth in a batch culture, in a batch operation. If we plot the cell concentration with time, the way the cell concentration varies with time during growth in a batch, that is we have dumped all the cells and provided the medium and so on so forth, staggering the process and we are monitoring how the cell concentration varies with time. The variation is typically like this. You have a certain time when there is not much of an increase in cell concentration. After a certain time, there is a significant increase in cell concentration, and then there is, it kind of flattens out, there is not much of an increase in cell concentration after it reaches its maximum value.

The period during which, the initial period during which there is no change, not much of a change in cell concentration is called the ‘lag phase’. The period where there is a significant increase is called the ‘log phase’, and the later phase where it tapers off is called the ‘stationary phase’. By the way, this is a typical batch growth curve. You may not always find it. Depending on the situation, the log phase may start immediately after inoculation. Or, it may take a very long time in lag phase, and then grow very quickly and then reach stationary phase, and so on and so forth, okay?

There are variations to the theme, this is a typical theme. And after it has reached a certain stage, if you are following the total cell concentration, it will probably remain (station), remain the same for a large period of time and then probably go down depending on the situation. Whereas if you are following the viable cell concentration, it is going to go through the same lag phase, log phase, stationary phase, and then there will be a decrease in the viable cell concentration. Now, let us do a mass balance on cells in the bioreactor broth. A bioreactor broth is what you are going to consider as a system.

The bioreactor broth is nothing but this, right? This is what we are going to take as our system, this is what we are going to concentrate on, this is what we are going to focus our attention on. If we do that, the basic material balance equation, this equation can be blindly written. We are following cells, therefore the rate of input of cells minus the rate of output of cells plus the rate of generation of cells minus the rate of consumption of cells equals the rate of accumulation of cells in the broth, bioreactor broth. Since this is a batch system, and we are going to consider only the growth now, let us not consider the death process here and so on and so forth, we will consider till somewhere here.

There is no input, because the batch operation, as I had said, we dump everything in, and start the time after everything is inside, okay? So there is no input stream into the system that we are considering. Similarly, there is no output stream. After the process is complete, we take everything and and go for further processing. And our period of interest now is the time when from start, after everything has been dumped in, to end before everything has been taken out. Therefore during that time of interest, there is no, there are no inputs, there are no outputs, and therefore the rates of input and output of cells is zero.

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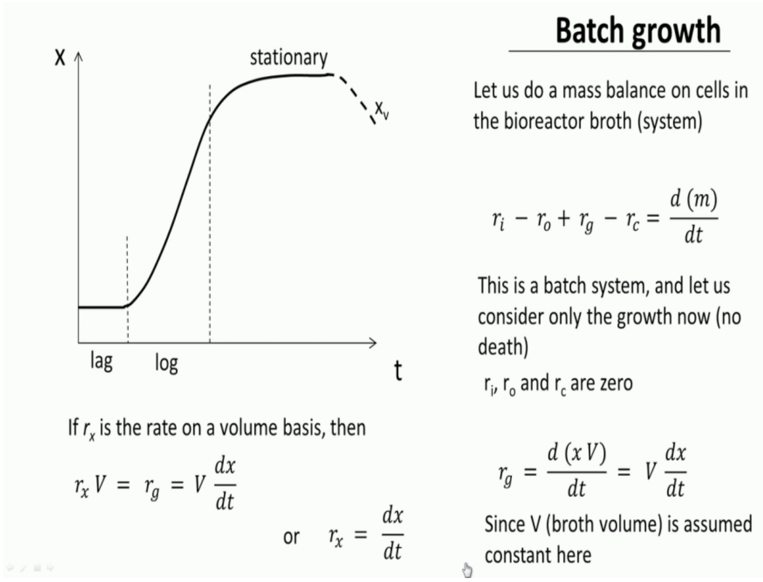


The rate of consumption of cells is also zero because the cells are not getting consumed by some means. They are only being generated. This is the case, then this, this and this (goes) go to zero, rg is the only term that remains and rg equals dmdt. We can write mass as nothing but concentration times volume, because concentration is nothing but mass per volume, right? So, concentration, given as x, x into the broth volume, this gives you the mass of cells, ddt of xV equals rg. In the case of a batch, the broth volume remains a constant, and therefore it can be taken out of the derivative here, and you get V dxdt.

If rx is the rate on a volume basis, which it usually is, then, we need to write rxV equals rg. rg, remember, is on a mass basis, right? It is mass per time, whereas rx is cell concentration per time. So cell concentration needs to be converted to cell mass, which can be done by multiplying it by the volume, rx into V equals rg, equals V dxdt. Or, rx, the V V gets cancelled out, rx equals dxdt. One of the most common models for growth rate that we have already seen is rx equals mu x, okay? Therefore, if you substitute rx equals mu x, we get mu x equals dxdt. Let us write as dxdt equals mu x.

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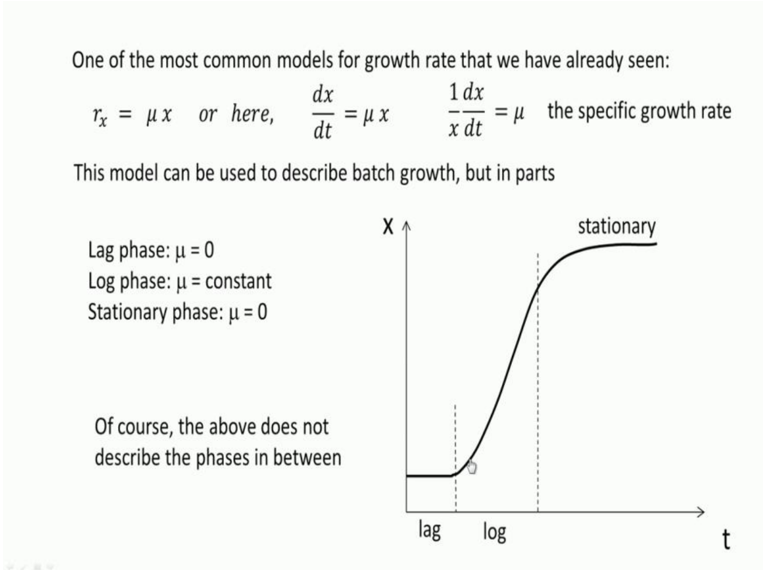


And one by x dxdt equals mu, it is easy to see why it is called the specific growth rate. This we have already seen earlier, where even in a more generic representation of rx. This model can be used to describe batch growth, but only in parts. The lag phase, the log phase, the stationary phase is what we had seen in a typical batch growth. In the lag phase, there is no increase in cell concentration over time, therefore the specific growth rate is zero. In the log phase, this you you will see immediately after the specific growth rate happens to be a constant. And, in the stationary phase, again, the specific growth rate is zero, because there is no increase in cell concentration with time. Right?

Among the various stages of growth, this phase, the specific growth rate and this phase happens to be the maximum, and therefore this is also called the maximum, the, the growth rate here, the specific growth rate here is also called the maximum specific growth rate in a batch, okay, that is another term that is used, and usually it is a constant here. But this model does not describe what is going on here when there is a variation, does not describe what is going on here, and so on and so forth. Therefore it does not describe the phases in between, okay? This we need to keep in mind.

But probably we do not need that, okay, all this is dependent on the need. Therefore we will realize the limitations of its representation, and use it appropriately. In other words, we cannot use it to represent things changes here, and changes here. Let us begin, or let us consider the log phase where mu is a constant. And we can write dxdt equals mu x. If we solve this simple first order differential equation, with the initial condition that a time t equals zero, the beginning of the log phase, the cell concentration is x0, okay?

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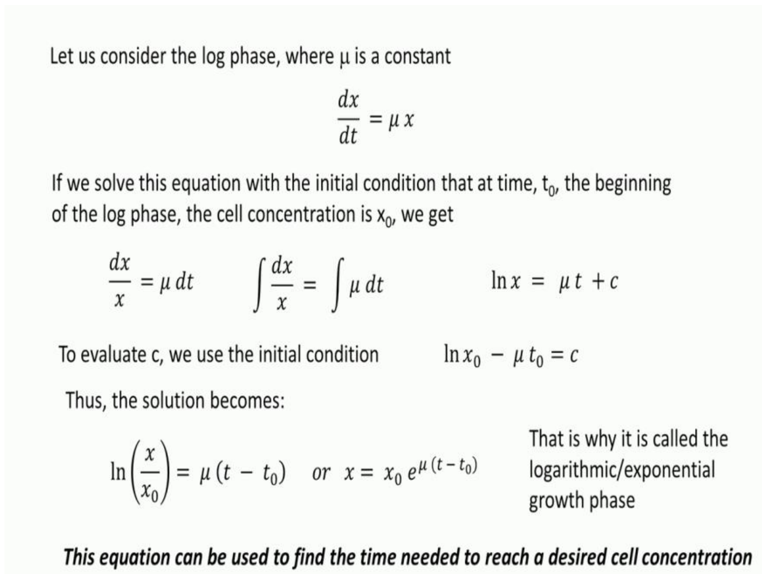


It is not the beginning of the batch, it is a beginning of the log phase, time t0, and the cell concentration is x0 at that time. If we do that, and we will solve this dx by x equals mu dt, integrating both sides, we get lon x equals mu t plus c. This constant can be evaluated using our initial condition, that at t equals t0, x equals zero, we substitute that lon of x0 minus mu t0 equals c, and therefore the solution becomes lon of x by x0 equals mu into t minus t0, or x equals x0 exponential mu into t minus t0. Okay?

And that is the reason why it is called logarithmic growth phase or the exponential growth phase. Logarithmic in this form, exponential in this form, and that is the reason why it is called so. This equation can directly be used to find the time that is needed to reach a desired cell concentration, starting from a certain initial cell concentration. And that is a very important design aspect. We would like to know what is the maximum cell concentration that needs to be reached, how long do you need to operate the bioreactor to reach that and so on and so forth apriori. Okay?

And if we know the specific growth rate apriori, this is a straight forward simple calculation to find out the time t that is needed to reach a given maximum cell a given cell concentration, okay? It is always important.

And of course, we are assuming that the cell concentration is less than the maximum cell concentration that is possible to achieve under those set of conditions. I think that is where we will sign off here, , we have seen culture growth or population growth, we have seen the need for quantification, insistence and the importance of sticking to time rates in trying to, when, when we quantify systems, dynamic systems, including biological systems, that is a cell, and then we looked at batch growth and quantification of batch growth through specific growth rate and in application of that, to find out the time that is required to reach a certain desired cell concentration starting from a certain cell concentration.

Batch growth happens to be the work horse of many industries, many biological industries, now even nowadays. See you later in another module. Bye.