Environmental Chemistry and Microbiology

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Module - 10
Lecture - 49
Microbial Growth - I

Welcome everyone to the next module. This is module 10 and the first 3 lectures are going to cover issues related to microbial growth. So, we have a 3-part topic over here and today's topic is lecture 49, and the first part of Microbial Growth.

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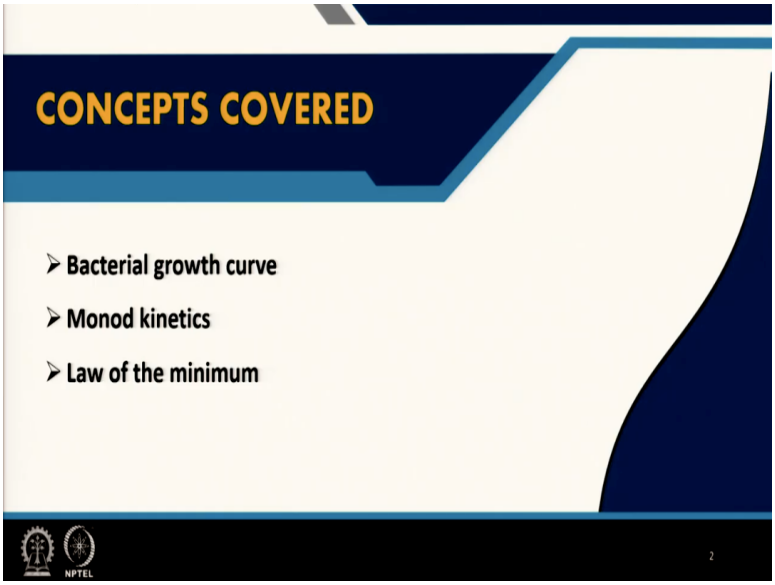

The topics that we are going to cover in this particular lecture is, we are going to look at the general or generic bacterial growth curve. We will derive Monod kinetics. We will go through the entire derivation and how it can be done, both mathematically as well as experimentally. And the last part is the law of the minimum.

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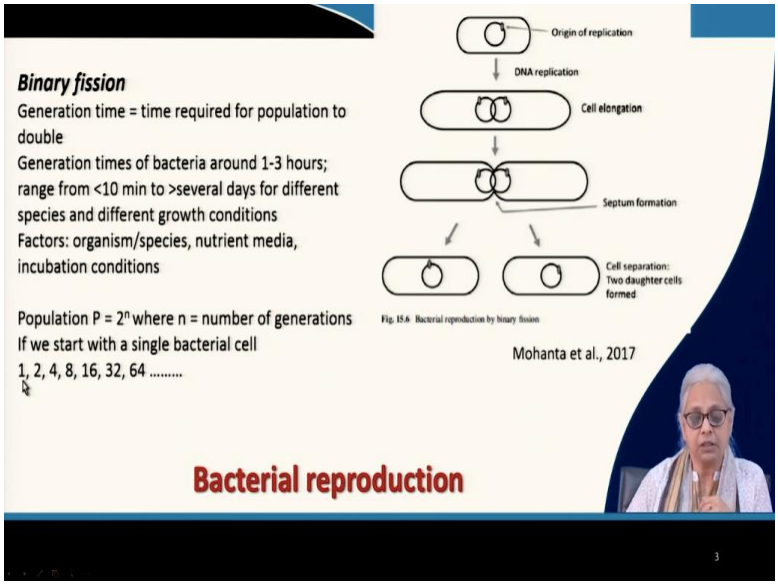

So, we are going to start with bacterial reproduction. And we already know, we have gone through it that bacteria reproduce by binary fission. Now, in binary fission, we have a single bacterial cell. So, that is what you see at the top over here in this graphic, and the cell has a double stranded circular DNA molecule. So, this DNA; the first step in the replication process or in the reproduction process is replication of the DNA.

So, this is what you see. There is an increase in the length of the cell and the DNA is replicated. So, we now have 2 circular strands. When the cell elongates enough, a septum will be formed. And you can see at the center, a septum has formed here. And eventually, the septum will lead to separation of the cell into 2 parts, with each part having its own double stranded circular DNA.

So, that is at the cellular level. Now, if we want to quantify the growth of bacteria in the lab or even in the environment, how do we do it? Let us go through some of the basics about it. The first thing is generation time. So, what is the time required for a single cell to become 2 cells? And these 2 cells will then become 4; and because each one will create 2, so one will go to 2; 2 will go to 4; 4 will go to 8; 8 will go to 16 and so on.

So, from the time that you have a single cell to the time that you have 2 cells, that is the generation time. That is the time required for the population to become double the original population. Now, generation times for bacteria can be around 1 to 3 hours, or if you take a wider range, it can go from less than 10 minutes to several days, depending on the species and depending on the growth conditions.

The same species under different growth conditions will have a different growth rate, and we will come to that later. So, what are the factors that affect the reproduction of bacterial species? The first thing is the species itself. Not all species, not all organisms will grow at the same rate.

So, different species, different organisms have different growth rates. So, it is species specific growth rates. That is the first thing.

Second thing is the nature of the nutrient media. If you provide it rich media, rich meaning high-end nutrient concentration, then obviously they are going to reproduce faster. If the nutrient concentration is low, they will reproduce at a smaller or slower rate. I will show you some schematics about that. And the third thing is incubation conditions. So, what are the environmental conditions?

What is the pH? What is the temperature? What is the pressure? All these conditions will determine the growth rate of the organism or the species that you are cultivating. If we want to put it in mathematical form, you can say population P; we use different terms; in different textbooks, you will find different terms. Sometimes the population of the bacterial species is shown as capital N, sometimes by capital P and sometimes, if you are measuring it in terms of biomass, it is shown as X.

So, all of these symbols are acceptable. So, we have population $P = 2^n$, where n is the number of generations. So, this schematic shows you 1 generation. Now, if 2 becomes 4, that would be 2 generations. So, like I said, if we start with a single bacterial cell, we get 1, 2, 4, 8, 16, 32, 64 and so on.

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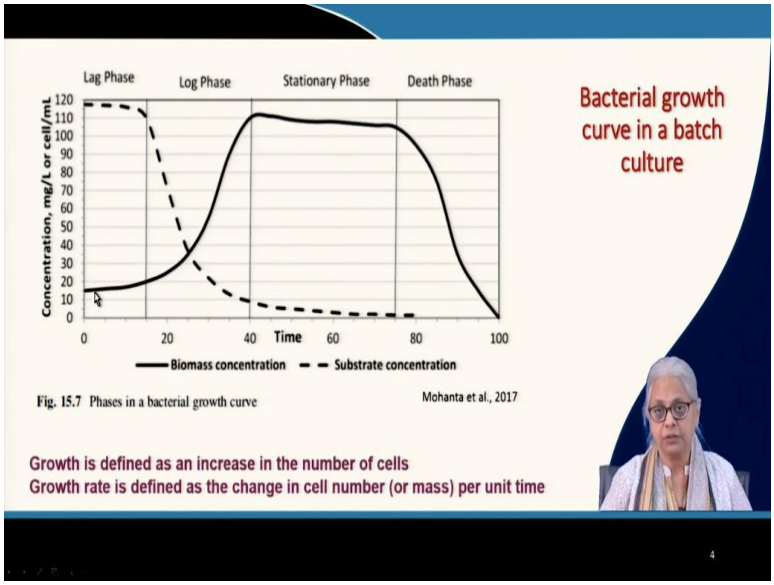

So, what I just said is the nature of reproduction in bacterial cells. Now, when we do experiments in the lab, what we generally do is, we take a batch culture, we add an inoculum to it, and then we monitor the growth of the bacteria. So, this is what I would call a generic bacterial growth curve. Now, how do we measure growth? The first thing is, what is growth? Growth is defined as an increase in the number of cells; and then we need to quantify growth

rate. So, growth rate is the change in number of cells over a period of time. And this is very important for whatever we are going to go through in the next few slides. Now, when you are doing this in a batch culture and you try to, let us say, draw a curve with the bacterial concentration in one case; and in the second case, if you are able to monitor the substrate concentration as well; then you will get 2 curves.

So, the dark black line in this schematic is your biomass concentration and the dashed line is the substrate concentration. I have been talking about glucose as the starting point. So, you can imagine that your substrate is glucose. It can be any other organic compound. Let me also add another word over here, and that is, the substrate is the limiting nutrient. So, you have to generally define or rather design your experiments on the basis of limiting nutrient.

And I will come to the idea of limiting nutrient when we talk about the law of the minimum. So, we will keep that for the end of this lecture. But for now, just imagine that the substrate is the growth limiting nutrient. So, in general, there is only 1 nutrient that is limiting the growth of the bacteria. And that is what we are going to be monitoring in these experiments. So, you have these 2 curves, one is the biomass and the second is the substrate.

You can measure concentration in 2 ways. You can measure it in terms of concentration of volatile suspended solids, or you can measure it in terms of numbers of cells. And the number of cells can be; if you are measuring it by microscopy, then it is total number of cells; if you are using plate counts, which we generally do, then you are measuring the number of living or reproducing cells. So, I will come to some of these details later on.

And that is your concentration on the y-axis. And on the x-axis you have your time. Now, biomass, as you can see, is increasing in this kind of curve where there is initially….. So, initially you will find that there is very little increase in cell concentration. Now, if there is very little increase in cell concentration, then what is the reason for that. So, remember you have started the culture. This is the beginning of the culture. So, the bacteria have to be acclimated.

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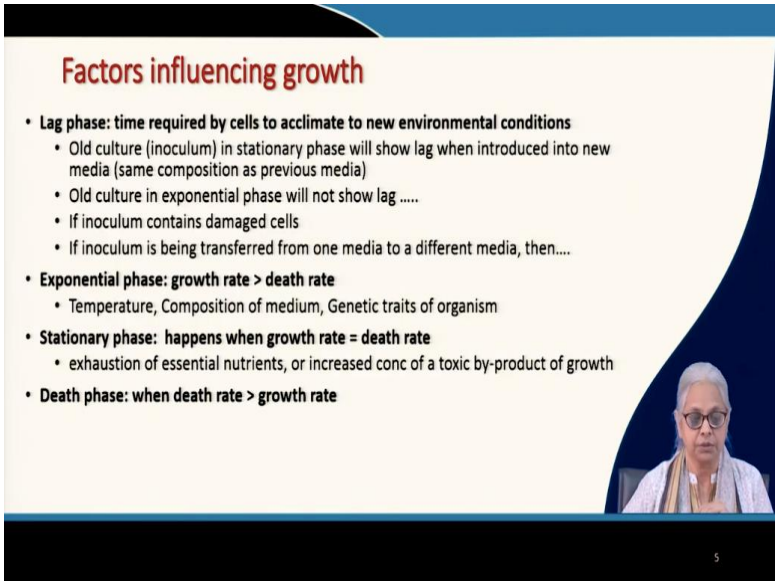

The time required by the cells to acclimate themselves to the new environmental conditions. You have taken them from one media and put them in a fresh medium. So, this old culture; if the inoculum is an old culture in stationary phase, it means they are already kind of not growing in their exponential (phase). We will come to all these points as we go along. It will show a lag period when it is introduced into a new media of the same composition as the previous one.

If the inoculum is in exponential phase, which means the second phase; this is the log phase; lag phase, log phase; log phase is also called exponential phase. So, if it is in exponential phase from the old media and you add it to the new media, same composition, same conditions, there will be no lag phase. So, the lag phase will be almost invisible. If the inocula has damaged cells; so, for example, if I am doing disinfection experiments, then the inoculum is somewhat damaged. It contains a large number of damaged cells and very few survivors. Now, it is those survivors that are going to reproduce. The damaged cells cannot reproduce. So, under those conditions, you are going to see a lag phase. You can also have a lag phase when you are transferring the inoculum from an old media which may be a richer media, and giving it to a new media which may not be as rich as the old media.

So, again the lag phase will be visible and quite clear. So, this is the lag phase followed by the log phase. Now, another hallmark of the lag phase is that the growth rate and death rate are more or less equal. That is why you see a more or less horizontal line. So, that defines the lag phase. There comes a point at which the number of cells that are there in the media have become acclimated to the new conditions. And then they start multiplying rapidly.

And there is no limit except the nutrient availability. So, that defines our log phase. Now, they are limited only by the growth limiting nutrient. So, at that point, the growth rate is much

greater than the death rate. Basically, when we talk about incubation or environmental conditions, first thing is temperature. If you change the temperature, the rate of replication will increase.

If you change the composition of the media, again there will be a change; and the genetic traits of the organism. So, different species will grow at different rates (under the same conditions). But in the log phase or the exponential phase, the slope or the growth rate is really dependent only on the growth limiting substrate concentration. So, that is the hallmark of the exponential or log phase.

Then we come to stationary phase. In the stationary phase, you will find that again the growth rate is more or less equal to the death rate, and you get a horizontal line. Now, in actual experiments, you may or may not see this. In my experiments with poor media, I did not really see a stationary part of the curve. It went up and then started going down almost immediately. So, it depends. The nature of the stationary phase can be very short or it can be quite long.

What does it depend on? It depends on the concentration of toxic byproducts in the media. So, let us say a large number of acids are created. For example, in aerobic media, you will find; in any media in fact, aerobic or anaerobic, a large amount of acids are created in the first phase of metabolism. So, the pH of the media will go down unless there is sufficient buffer added to the media.

Now, if there is insufficient buffer, then you will find that the pH has gone down. And you will also find that the essential nutrients are being exhausted, because the biomass has increased to such a point that there is insufficient nutrient availability. So, these are the 2 major conditions that will determine the nature of the stationary phase, how long is it or is it visible in your experiments and so on.

So, those are some of the things that you can look for. And finally, we come to the death phase. The death phase is when the death rate is much greater than the growth rate. So, at this point, when the death rate is higher than the growth rate, the cell concentration starts coming down. And it can come down very fast at an exponential rate or it can come down very slowly.

So, again it depends on the environmental conditions or the incubation conditions, as well as the nutrient availability and the concentration of toxic by-products. So, all these factors will determine the nature of the curve. But this is what a generic bacterial growth curve looks like in a batch culture.

Look at the substrate concentration. The substrate concentration is the reverse of the biomass concentration. In the lag phase, there is no change, which means there is no significant utilization of the substrate in the lag phase. As the exponential phase takes off, the substrate

consumption is very fast as well, because the biomass is utilizing the substrate, and you get exponential decay in the substrate concentration in the media. Stationary phase: Again, it is more or less horizontal.

And in the death phase, you will find that most of the substrate has been consumed in and become part of the biomass, and you have very little substrate left towards the end. Now, in some cases, this may never happen; this may never be 0; it may stop at some other point anywhere in between the time = 0 concentration levels. So, S at t = 0 is the highest concentration. And you may have, you may never get to 0; it all depends on the nature of the experimental conditions.

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Now, what we want to look at next is the exponential part of the growth curve. So, let us just look at our generations, time and number of cells. Now, you can express the number of cells like I said, either in terms of cells per ml, or in terms of milligrams per litre, and we measure it as volatile suspended solids. So, it depends on what you are trying to do, what you are trying to achieve.

So, you can do it anyway you want to; that there are several methods for doing that. Now, our first generation, and assuming our starting point is a single cell. So, the first cell is 1. It goes to 2, 4 and so on. So, this is what you see over here over a period of time. And assuming that the doubling time for this particular hypothetical case is 10 minutes. So, here you have the time, the increments of time.

You have the number of generations and the number of cells. So, the number of cells is growing exponentially and that is shown by the orange curve; and the blue line tells you the generation number. So, that is the number of generations; and the orange line tells you the number of cells.

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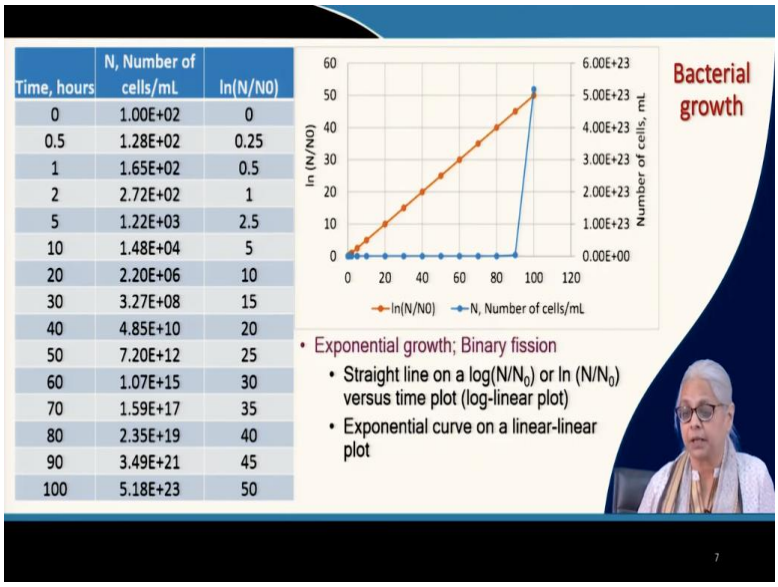

Now, we do not usually start any experiment with a single bacterial cell. It is not possible to inoculate any media with a single cell. We generally start with (an) inoculum of maybe thousand cells, million cells and so on. Now, here we have another hypothetical example where you have time in hours. The initial concentration is 100 cells per ml. You can convert it to ln $\frac{N}{N_0}$. N_0 is the number of cells at t = 0.

So, when $N = N_0$, that is our starting point, at t = 0. So, these are the graphs that you are going to get. The orange line is the log concentration. So, log N by N 0. So, this is what we also call normalized cell concentration. So, you can write it as natural log; you can write it as log base 10; either way you know how to convert from one to the other. And you have ln $\frac{N}{N_0}$ is a straight line.

So, on the x-axis, we have time which is not mentioned here; but this is time on the x-axis. And you can see that the number of cells is very difficult to show on a linear scale, because they are growing exponentially. So, you need a log scale to be able to show the number of cells and the relative growth relative to the initial concentration. So, we call it normalized cell concentration.

So, this is natural log or you can write log base 10, either way. This is what we call the exponential growth phase. It is by binary fission. You get a straight line on a $log\ \frac{N}{N_0}$ plot or on a natural log $\frac{N}{N_0}$ plot. So, this is a log-linear plot, which will give you a straight line. And on a linear-linear plot, it is very difficult to look at it, because your numbers are very high.

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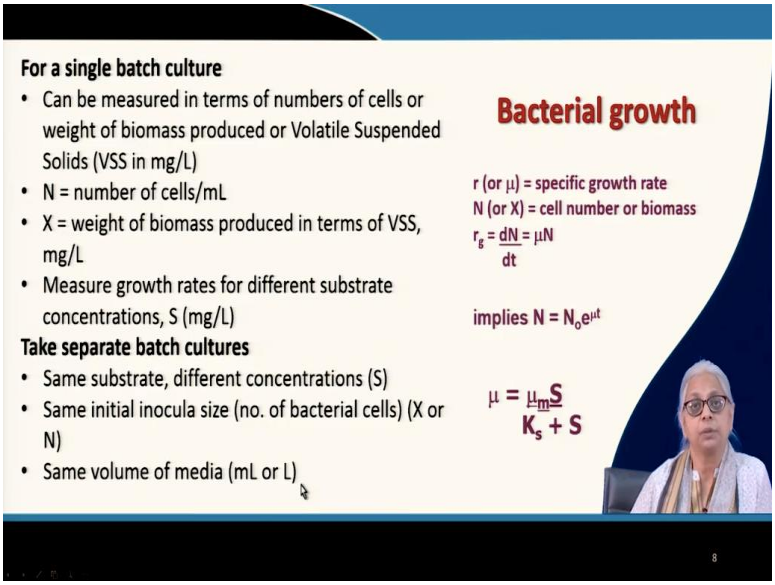

So, here we come to what happens in a single batch culture. Now, in a single batch culture, what you will be doing is, you will be measuring the number of cells or the weight of the biomass produced. Now, if you are measuring weight of the biomass, you will generally filter the media. Take a small amount of the media; filter it; measure the volatile suspended solids. So, that is VSS; and we normally express it in terms of milligrams per litre.

Number of cells: If you are doing plate counts or microscopy, you will do it in terms of number of cells per ml. So, there are different ways of doing it. So, N normally stands for number of cells per ml, X normally stands for the weight of biomass produced in terms of VSS. You also want to measure growth rates, and we will come to the derivation of Monod kinetics.

So, for deriving the equation for Monod kinetics, a single batch culture is not sufficient. For that, what you need is the same growth-limiting substrate, but different concentrations of the substrate. So, you will do a series of experiments with different substrate concentrations. The same initial inoculum size, the same incubation period and incubation conditions and the same volume of media have to be maintained, so that everything else is the same except the substrate conditions.

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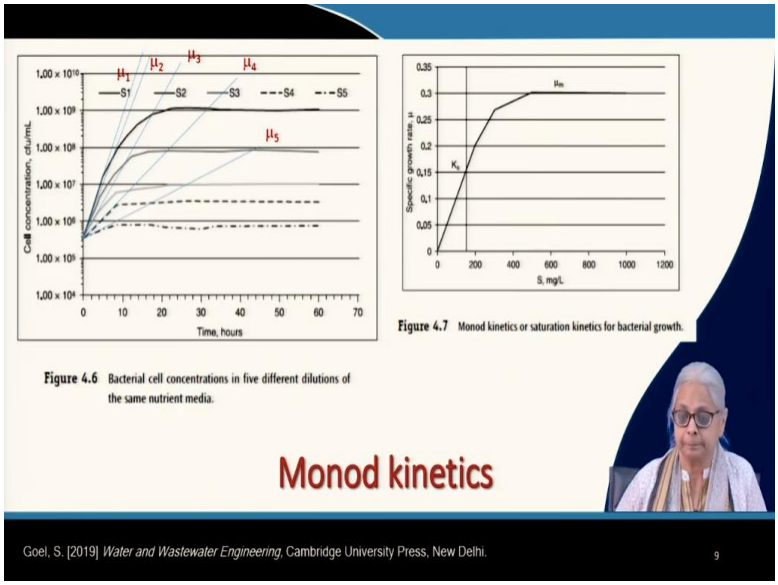

So, now here is how we come to measuring the parameters required for quantifying growth and for understanding Monod kinetics for any growth limiting substrate. So, as I said, you run a series of experiments with different substrate concentrations; and the substrate is your growth limiting nutrient. So, here you see 5 different substrate concentrations, S1 to S5. S5 is the lowest concentration and S1 is the highest concentration.

On the y-axis, you have the cell concentration; and on the x-axis, you have time in hours. Now, when you run each of these batch cultures, you are likely to see the curves that are shown in this graph. At some point, you will find that they reach stationary phase. Now, we are not really interested in the stationary phase. The stationary phase and the concentration at the stationary phase level is a function of the substrate concentration.

So, we will come to that point in a little bit. What is important in the exponential phase is the slope of each one of these curves. So, we are going to call the slope of each curve μ_1, μ_2, μ_3, μ_4 and μ_5.

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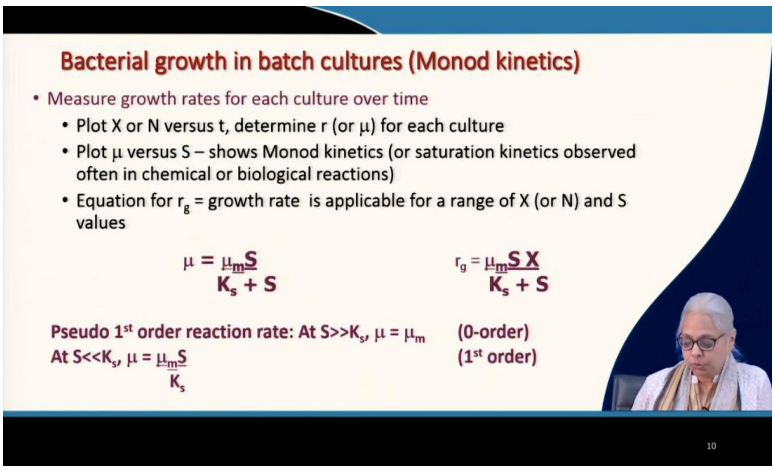

Now, that is what I have here. r or mu is the specific growth rate; And that is particular for a specific substrate concentration. So, S1 corresponds to μ_1, S2 corresponds to μ_2 and so on.

N or X is the cell number or biomass.

And when we write the generic form, the growth rate; r_g stands for growth rate; you can express this as $\frac{dN}{dt}$ or $\frac{dX}{dt}$. If you are using the number of cells, then $\frac{dN}{dt} = \mu N$; and when you integrate it, you get $N = N_0 e^{\mu t}$. Now, as I said, this mu itself is a function of the substrate concentration. So, we want to come to this equation. Now, let us see how we get there.

We now have a series of mu values and we can plot them against S. So, we have 5 S values and corresponding μ values. So, when I plot μ versus S, I will get a curve like this; I should get a curve like this. Now, there is a point at which you get maximum growth rate. Regardless of what your S value is, you can continue increasing S beyond this point, the μ value will not change. So, that is my μ_{max}. So, this is μ_{max}.

Then we define another parameter called K_s. K_s is defined as the half-velocity constant, which is the substrate concentration at which the specific growth rate is exactly half of μ_{max}. So, when μ_{max} is half; so, here we have $\mu_{max} = 0.3$; half of that is 0.15; what is the corresponding substrate concentration? And that is what you see over here. And that value is K_s. So, having gone through these 2 sets of data acquisition you might say, then we come to how we derive this equation.

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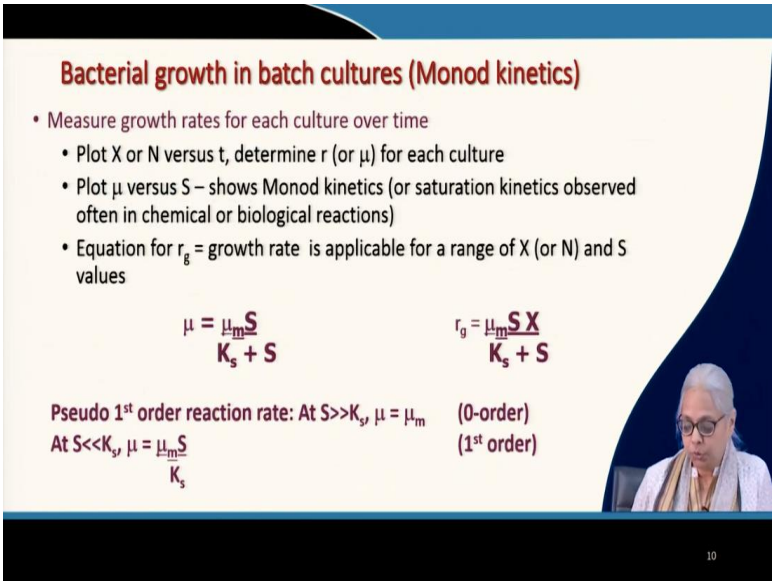

So, now that I know that μ is a function of S, I can write it in this form, which is called the pseudo first order reaction rate. Now, when S is much greater than K_s; so we have K_s over here, and let us say S is much greater than K_s. So, in this region, we see that $\mu = \mu_{max}$. So, it is a 0-

order reaction over there. Then we have another case where S is much less than K_s. So, let us say we are in this part of the curve.

At this point, it is a first order reaction, because if S; you can do the math yourself; and if S is much less than K_s, then it drops out of this equation; it can be thrown out literally; and you get $\frac{\mu_m S}{K_s}$. So, this is negligible compared to K_s, and you get $\mu = \mu_m$. So, this is your first order. And otherwise, in the intermediate 2 situations, this is our equation which holds for all of them. Now, we need another parameter to relate substrate to biomass. So, what is the relationship between substrate and biomass?

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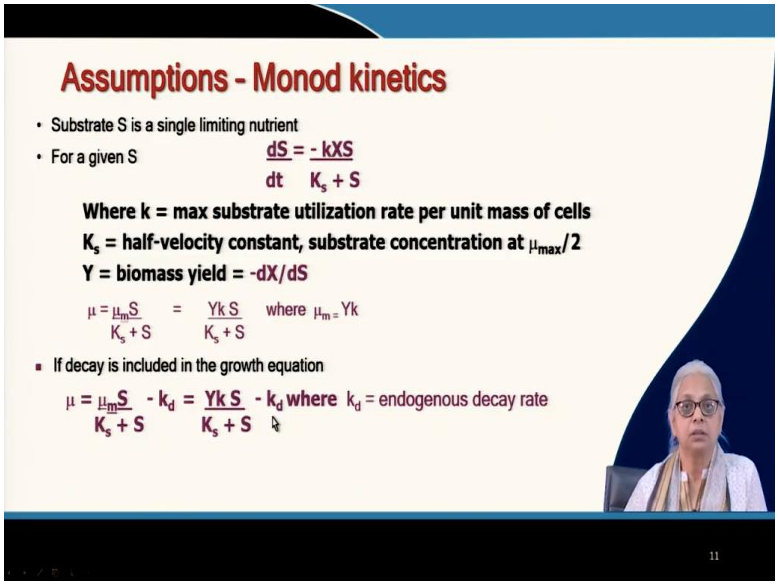

That is given by what we call yield. So, biomass yield describes the relationship between biomass and substrate. So, we have - dX/dS tells us the biomass yield and it also gives us a relationship between biomass and substrate concentration. So, what are the assumptions in Monod kinetics? Now, we know that our substrate is a single growth limiting nutrient.

For any given S, $\frac{dS}{dt} = \frac{kXS}{K_S+S}$. Now, what are these two new parameters? k is the maximum substrate utilization rate per unit mass of cells. So, instead of monitoring number of cells; up to this point, I have been talking in terms of monitoring the biomass or the number of cells. Now, instead, if you have no ability to monitor the biomass, and instead you are monitoring the substrate, which happens very often in the kinds of experiments we do; then you have a k value. So, this is your maximum substrate utilization rate per unit mass of cells. I have already mentioned what K_s is; that is the half-velocity constant. The substrate concentration at $\mu_{max}/2$. And biomass yield has also been defined. Now, given what we derived in the previous slide,

we can now substitute these values. So, Y can be expressed as μ_m by small k. That will give you the conversion factor for going from X to S.

And μ_m remains the same, as I said it. And then, μ_m will be substituted here by Y_k; and you get $\frac{YkS}{K_s+S}$. Now, why this form? Because this is easier to measure. So, Y can be measured easily. You know how much substrate has been consumed. The initial and the final concentration is given. And you know how much biomass is produced. So, Y is one of the easiest parameters to measure.

And small k, like I said, is also not difficult to measure. It depends on the nature of the substrate.

Then we come to the last part of this derivation. I have already shown you the generic bacterial growth curve; and we know there is a death phase or another word that is used is the endogenous decay phase. In cell biology, I spoke about the fact that you have feast and famine type conditions.

So, when the nutrients are exhausted, the cells are in a famine like situation. The nutrient availability is very low compared to what they require. So, they enter into this death phase or what is often called the endogenous decay phase. They will utilize whatever storage granules they have; they will consume their own biomass; and that is why it is called endogenous decay rate.

And there is no further growth, because they cannot reproduce; the nutrients are not there; there is insufficient nutrition for the cells to reproduce and continue to increase. So, this is the final stage in the growth curve. And μ at this point is modified to include the endogenous decay rate. So, this is - k_d. In some textbooks, the letter b is used; and we will be seeing that in a subsequent lecture. So, either way, it remains the same, it means the same. So, this is the same equation with the final stage, the endogenous decay rate is included.

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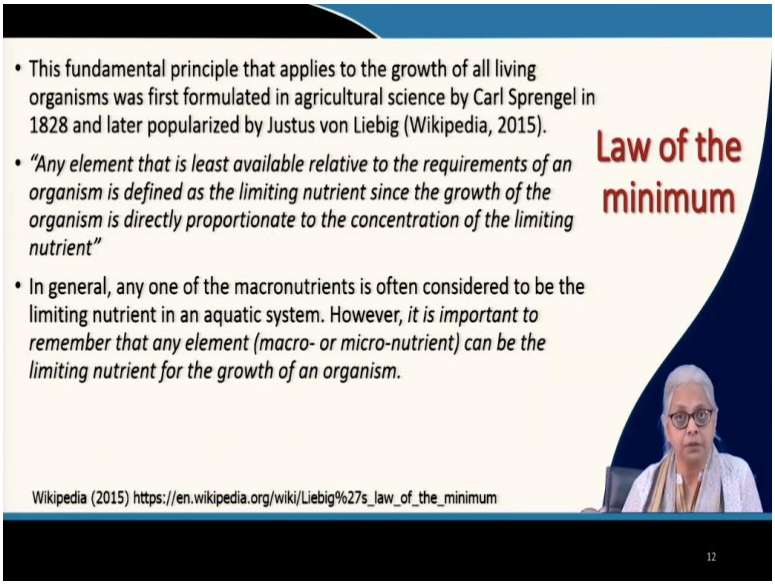

Let us now come to the last part of this topic, and that is the law of the minimum. I have been talking about the fact that there is a growth limiting substrate or a growth limiting nutrient. Now, we have seen that all bacteria as well as other organisms require not just one nutrient, but many different nutrients, may be hundreds. How do we know which one is the growth limiting nutrient?

So, this is the law of the minimum that helps us to define the growth limiting nutrient. This is a fundamental principle that applies to the growth of all living organisms. It was first formulated in agricultural science, way back in 1828; and it was popularized by Liebig. So, many textbooks, the older textbooks use it; and they often call it the Liebig's law of the minimum. But this is some new information that has come to light.

What is this law? Any element that is least available relative to the requirements of an organism is defined as the limiting nutrient. And the growth of that organism is going to be directly proportionate to the concentration of the limiting nutrient. Any of the nutrients that we have seen in previous lectures can be a growth limiting nutrient. But for simplicity, because it is very difficult to do complex things; everything that we do in the lab and the assumptions we make for environmental conditions are often very simplistic.

And we assume that one of the macronutrients are the growth limiting nutrients. So, we can say, it is a carbon limiting growth media; we can say, it is a nitrogen or a phosphate or a sulphur limited growth media. We generally say these things about our experimental conditions. However, it is important to remember that any of the elements, they may be macronutrients or micronutrients, any of them can be the growth limiting nutrient for any organism.

It is also important to remember that when we talk about nutritional deficiencies, even within human beings, it is a same law that we are keeping in mind. Because, it is the particular nutrient that is deficient in either the diet or the environment of that person and or any other organism, and that is what is causing some impact on the growth of that organism; it may be a human being or any other organism.

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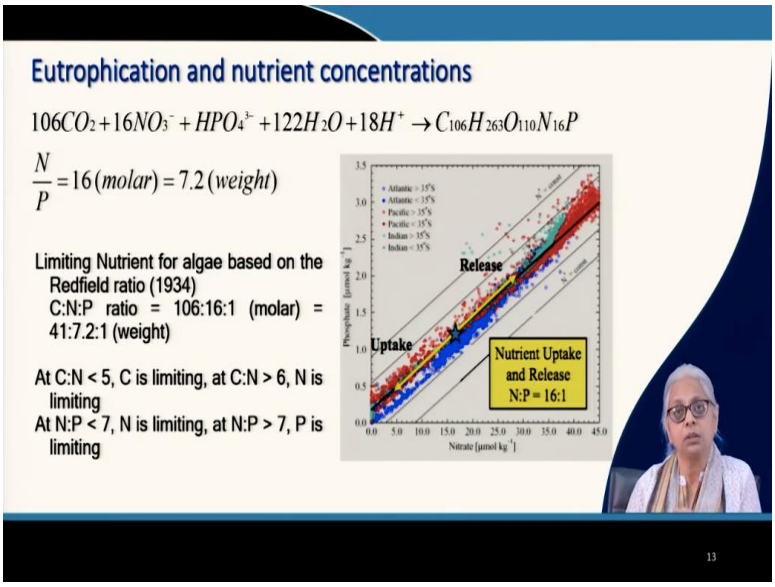

Then we come to eutrophication and nutrient concentrations. Now, what happens in eutrophication is that you get algal production as a function of carbon dioxide, nitrogen, phosphorus; and I have kept it as simple as carbon, nitrogen and phosphorus. Now, these are 3 essential macronutrients that are required for the growth of something like an algae. You can do this for bacteria; you can do this for any other organism; the same principle applies.

What does this empirical formula for the algal cell tell us? This is the molar ratio; so, this is the stoichiometric empirical formula for an algal cell. For a bacterial cell, we use $C_5H_7O_2N$, and that is available in all the textbooks. So, you can use the same principles to understand the same idea and apply it to a bacterial cell. I am taking a more complex example here, of the algal cell.

So, we have taken 3 essential nutrients: carbon, nitrogen, phosphorus; assuming that hydrogen and oxygen can never be limiting, because these organisms are growing in water; and water is plenty, so they will never be limiting - hydrogen and oxygen. So, we focus on carbon, nitrogen and phosphorus. So, ignoring carbon for the first part, we have nitrogen to phosphorus ratio on a molar basis is 16.

And when you multiply it by the molecular weights, it becomes 7.2. Then we come to the second part. Let us put all 3 of them together: C, N, P. So, what is the ratio of carbon, nitrogen, phosphorus? That is 106 to 16 to 1. Now, notice that the last element, the least required element is 1. So, everything else is normalised with respect to phosphorus. So, you can convert it.

Either, you can utilise either moles or milligrams or grams, whatever is acceptable. And this brings us to what is called the Redfield ratio. The Redfield ratio is about the nitrogen to phosphorus, but it also includes several other nutrients. If you go back to the original paper, you will find that it includes sulphur and silica and so many other elements. But people have generally focused on nitrogen and phosphorus.

So, what was interesting about this particular paper which was published as far back as 1934 is that the phytoplankton in all oceans, no matter which part of the world you are in, the ratio of nitrogen and phosphorus in the phytoplankton, which means algae for the most part, is fixed at 16 is to 1 molar ratio. And you can see, the Atlantic Ocean, Pacific Ocean, Indian ocean; you can see the data, it fits along this line of 16 is to 1.

So, this is nitrate on the x-axis and phosphate on the y-axis. And you can see that the uptake and release is more or less in equilibrium. And that is why it is called the Redfield ratio, for the person who came up with this idea and it was published and so on. So, this is a well-known concept that the rate of nutrient uptake and release, and these are the growth limiting nutrients for the algae or phytoplankton in the oceans and so on. And it is completely uniform all across the globe. So, with the carbon to nitrogen ratio less than 5, C would be limiting. At C:N ratio is greater than 6, N would be limiting. For N:P less than 7, N would be limiting; and the other way round, phosphate would be limiting.

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This brings me to the end of this topic. I will stop at this point. Thank you.