Applied Environmental Microbiology

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Lecture – 07
Microbial Energetics I

Hello students. In the previous lecture, I have introduced you to different proteins how they behave and then we talked about cells growth. So, how can we humans know where the cells are in their replication process how fast they are growing? Today I would like to revisit their model for cell replication because I think it will help gain some extra clarity about what is happening in the when the cells are growing.

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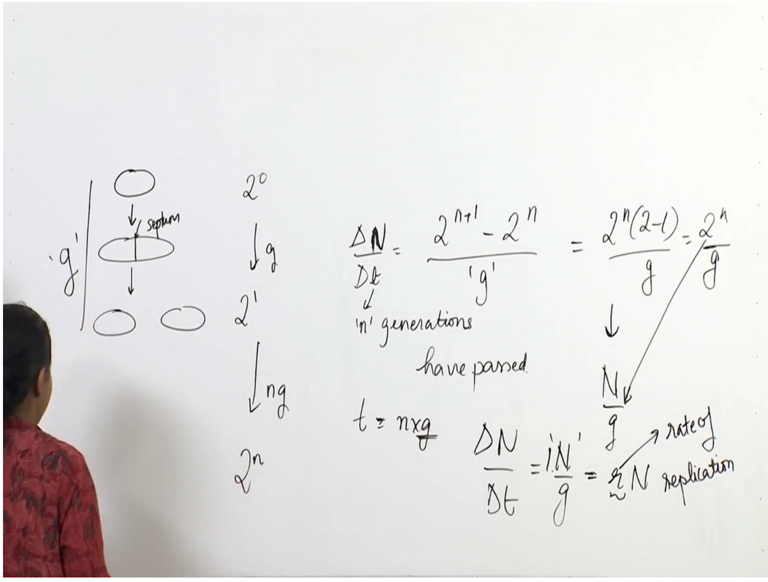

So, as a recap in the previous lecture we talked about how cell undergo binary fission. So, this is a typical cell and it will elongate create a septum.

So, septum is that place from which it starts staring into 2 cells and then low and behold within one generation time we will have 2 microbial cells, 2 daughter cells, where there was only one parent cell. So, we noticed that to begin with we had 2 to power 0 cells and then 2 to power 1 after 1 generation time and then after N generation times we will have 2 to power N cells.

Now in order to model this cell growth and I mentioned it previously one way we can go about is take a general time when N generations of a time has elapsed and find out what that typical microbial rate growth would be which is depicted by del N by del t, here n is a number of microbial cells t is the time and remember we started with n naught or 1 cell.

And here we can say that n generations have passed, what it means is that the time in this moment is actually n into g where g is the time it takes for one microbial cell to replicate under ideal conditions. So, del n by del t at this particular time when t is equal to ng the number of microprocessors that we should have ideally will be 2 to power 2 to power n and in within next generation time after time g in an interval of time g we will end up having 2 n plus one and thus del n by del t will be equal to 2 n plus one minus 2 n by g.

Now; obviously, this can be very easily solved by taking 2 to power n common and then we have 2 to power n 2 minus 1 upon g and thus we are left with 2 to power n upon g. Now notice here 2 to power n which is the numerator of this right hand side of this model is actually the number of microbial cells if we ideally have at this particular time already and thus we can also write this as N by g right.

So, to simplify now our model looks like del N by del t at any given time is equal to N by g which is number of microbial cells upon generation time correct. Now one upon g can be one upon g we will give it a different constant I mean we can write it as a different with different notation and refer to is r N, now note here g is the generation time thus g is the time it takes for cell to replicate it is reciprocal would give an idea of how fast cells are replicating; an example is if it takes you 3 hours to do a homework and it takes me to do 5 it is to do the same homework it takes me 5 hours.

So, I take 5 hours to do a homework you take 3 hours to do that same homework if we do reciprocal of our time that it takes for us to do the homework, we will get an idea of who is faster in doing the homework for me it would be one upon 5 and for you it would be one upon 3 and very easily we will know who whose rate is faster your rate will be much faster than mine. And thus r is also referred to as rate of replication and as a teaser I asked you to tell me; what is one major obvious limitation of this model.

So, today I want to visit briefly the limitation the major limitation of this particular model.

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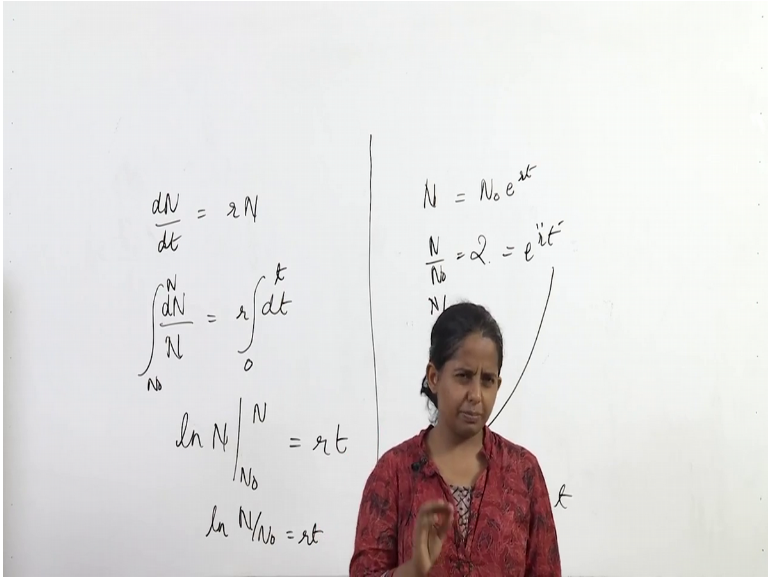

So, right now we have the growth model for binary fission of microbial cell as dN by dt is equal to r N. So, let us integrate this to get our equation for microbial growth and in order to integrate this we need to separate our differentials first. So, we can bring dt here and we are left with d N by n is equal to r dt. Now integrating from time 0 to t and N naught to N microbial cells now remember at time 0 we started with N 0 microbial cells at time t we have n microbial cells.

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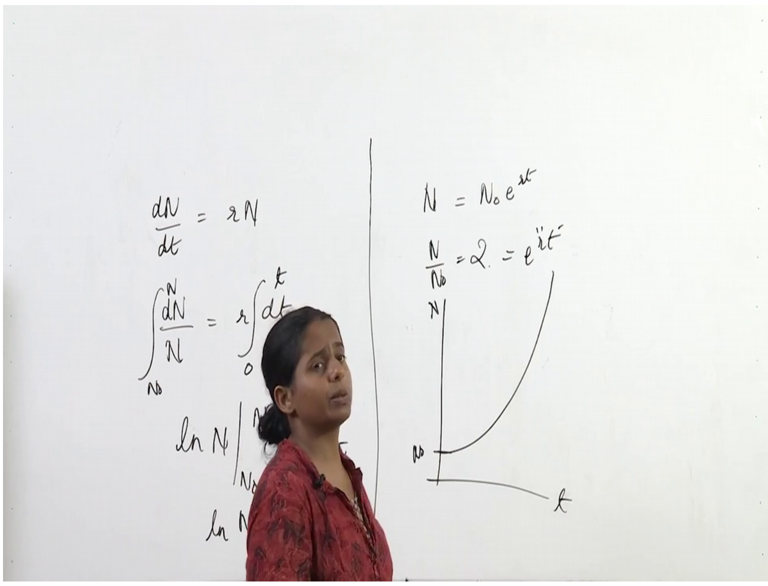

So; obviously, this is a natural log and here we have rt and thus we know, now that number of microbial cell at any time will be equal to initial cells that we started with into e rt. So, this is an exponential growth now with any exponential model whether it is exponential growth or exponential decay as this case with radioactive materials we have a concept of half-life or in this case because it is a growth model we will have a concept of doubling time.

So, how much time does it take for microbes to double and it should be very easy for you because for doubling time you would know that N by N 0 will be equal to 2 will be equal to ert and if you know the rate you can easily calculate the time.

Or if you know the doubling time because you have a mechanism for counting microbes and we talked about that mechanism then you can actually find out the rate. Now if you plot this model it looks like this you start with N 0 and then you have an exponential growth. So, as you increase time you will notice that microbial cells will grow very large a number very soon. In fact, there is a particular strain of e coli which is at predicted that if allowed to follow exponential model with just few microbes in 48 hours it will have more mass than the entire earth together which is; obviously, improbable and impossible completely impossible.

So, there is a major limitation with this model which is that it expects that as microbial populations increase nothing will change in the environment, but we do know that as number of microbes and their density increases in our media or in our environment the resources reduce and when the resources reduced another factor comes into picture. So, they are they are not just replicating, but they are also dying because of competition and lack of resources and thus in our model we have a need now to add another term that accounts for this decay. So, let us do that.

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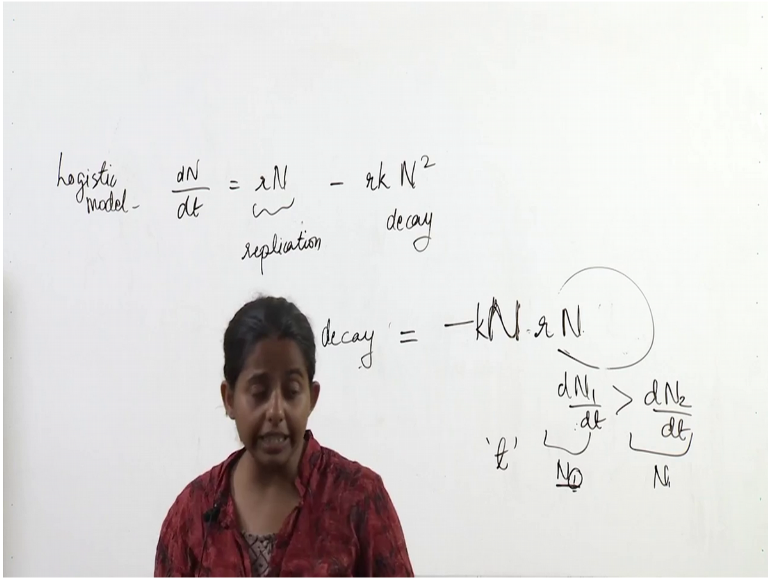

So, our original model look like this d N by dt is equal to r N right and this is our growth term and as I mentioned just now we need to add a decay term. So, in order to add a decay term let us think about it what will our decay depend upon a decay will I know it will depend on 2 things, but let us explore what it will depend upon let us say we have 2 microbial communities and at any given time one has N 1 number of microbes and the other have N 2 number of microbes at any given time t and they have exactly the same resources.

But you know does that N 1 is much larger than N 2. Now tell me which one will have more decay going on in their microbial community if they have exactly the same number of resources, but N 1 is larger intuitively we know that N 1 will also face a higher a number of decay in their microbial population and this is actually what we have observed in lab. So, we do know that this decay term has to be proportional to number of microbes present in the cell.

Now let us look at another instance when both microbial communities there are 2 microbial communities having exactly same resources one has N 1 number of cells and the other also has exactly same number of cells; however, there is a difference N 1 is growing much faster than N 2. So, N 1 is consuming resources at a faster rate than N 2 right in our current and more common day life we can draw an analogy that if there are 2 cities both have exactly the same population and exactly the same resources, but one of them is consuming the resources that they are endowed with much faster than the other.

So, tell me which city is headed towards destruction faster and we know that if d if N 1 microbial community is consuming resources faster than it is decay will also be faster. And thus we know the decay should also be proportional to d N by dt. Now because it is a decay it should not have a positive sign because positive will add to population we want to reduce the population. And hence we will have a minus sign here and in order to remove the proportionality, here we will add a proportionality constant let us make it k.

So, minus k N d N by dt. Now this d N by dt is the rate at which it is growing not the rate at which the population is having it is growth remember in biology growth includes both replication rate and decay rate. So, technically this is a replication rate. So, d N by dt would mean the replication rate can be replaced by this first term r N. So, now, we have our decay term minus kr N square and this is our new model and many of you might be aware that this model is very popularly called as logistic model it serves really well for simple populations that actually undergo either binary fission or have other similar comparable simple process of replication.

But whenever we start talking about insects that have a very complicated life style for example, mosquitoes some part they need to spend in environment, some part they have they have to do and deal with other hosts not host sorry they have to deal with other mammals and animals and then we have some other insects that actually require to live within a host such as many worms that live in our intestines at times. So, they have very complicated life cycles and we notice that with them the logistic model does not hold true.

But for simple growth such as microbial growth logistic model is pretty nice. Now let us try to understand what is model logistic model is. So, here we have d N by dt is equal to r N minus rkN square.

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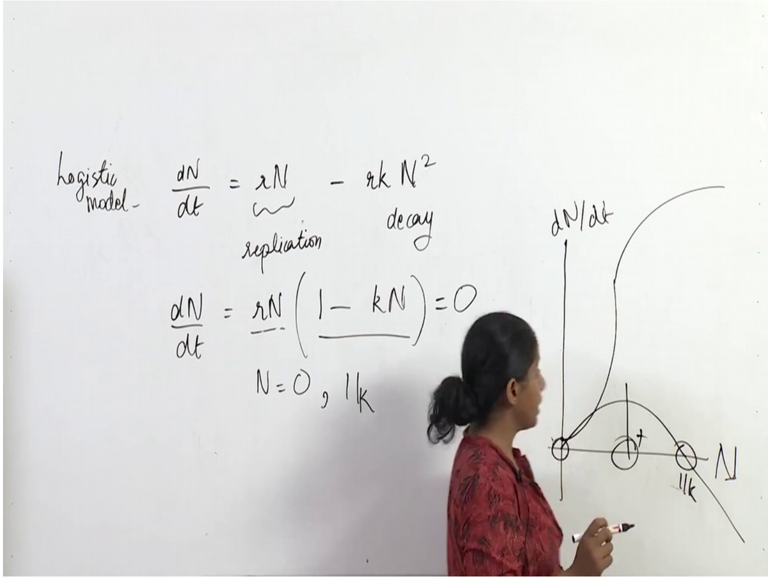

We can write it as r N 1 minus k N. Now this is a differential equation an ordinary differential equation and in order to in it is first order ordinary differential equation, in order to interpret these one of the easiest way is to make d N by dt equal to 0 and then use a geometric approach we do not need to study that, but many of us perhaps are engineers and because and some maybe we are not, but in any case I think it would be nice idea to revisit geometric approach of understanding these models because we do not need to integrate it.

And it is a good idea to understand of how the model is actually related to what we are observing in life. So, we have d N by dt if we make it equal to 0 this equation will be equal to 0 only when n is equal to 0. So, if n is equal to 0 this term becomes 0 or N is equal to 1 upon k when this term becomes 0 all righty. So, if we make a diagram with y axis being represented by a rate at which population is growing and x axis at number of microbes that are present at any given instance we know that 2 important points will be 0 and one upon k because at this point d N by dt will be equal to 0.

And a very quick understanding will tell us that r is always positive right because g is always positive time is always positive. So, one upon g equal to r is also positive and k is also positive and thus this will always be greater than 0 this can never be negative as long as N is between 0 and 1 upon k this will be positive too. So, we know that between 0 and one upon k this function; however, it looks like is positive and we also know that once it N goes above one upon k it will go negative; obviously, population cannot be below 0. So, we can actually rule out any analysis for n less than 0.

So, here we know that the rate of population growth up to some instance between 0 and one upon k will be in a positive direction. So, the slope here would be plus and thus we will see a u shaped growth and once it crosses this particular point we do not know what that point is here, but once it crosses this point then the d N by dt will start reducing. So, we will see your growth this years because it is still positive we will still see a growth, but the growth will slow down and we it will look like this and then once population reaches one upon k it will stop moving.

So, once the population has reached one upon k it will stop moving and graphically we can now interpret this data as.

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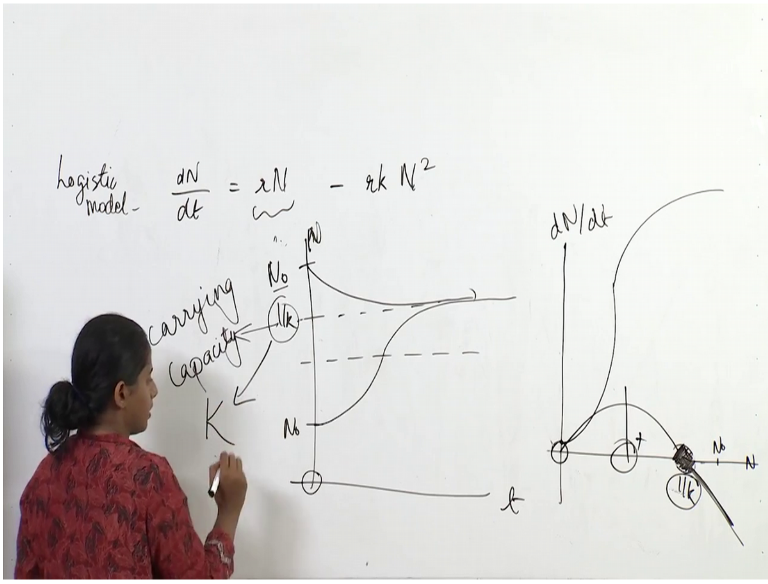

So, let us plot here this is time, this is N and this is 1 upon k and we are starting with N 0 which is much smaller than one upon k all righty. So, up to a certain point we do not know what that point is, but of course, we do not need to know right now up to a certain point the population growth will be like an inward like a u shaped curve and as it starts approaching one upon k this is how we will observe it.

So, we without solving the equation we already know how microbe macros will grow for a given N 0 now because the population here stops at one upon k, we know that population will not go above one upon k, but what if we start with microbial cells that are more than one upon k let us say our n naught is much bigger than one upon k. So, here when N 0 or our beginning N 0, let us say here is much bigger than one upon k which is this particular point we know that the growth rate would be negative. So, this is n naught let me make this more clear here this is N axis and this is N 0.

So, at N 0 we know that d N by dt is negative and hence instead of growth we will have a decay and we do not know how it would be, but the decay probably would be like a a u shaped curve left side of a u shaped curve. And thus we know we will decay until we reach one upon k and once we reach one upon k the population will stop reducing and it would not grow either and thus we notice that one upon k is quite a stable place for a microbial population to be it neither wants to wants to be greater than one upon k nor less than one upon k.

So, if N 0 is greater than one upon k it was stabilize there. So, we notice that as long as we do not have no microbial cell because if you do not have any microbial cell to begin with we cannot create microbial cells where no spontaneous generation here as long as we have any number of microbial cells sooner or later we will all end up at one upon k population. And thus one upon k is referred to as a carrying capacity of the ecosystem and we can give it a new notation a capital K. So, k is the carrying capacity.

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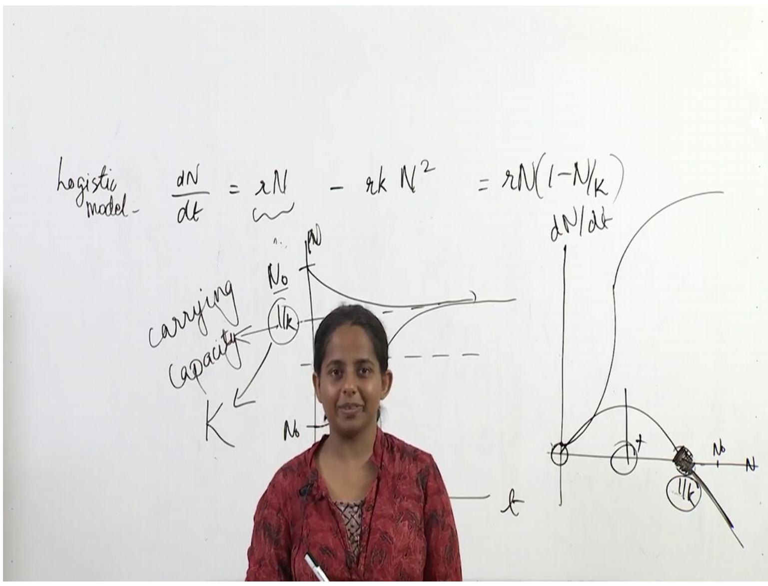

So, in our model we write it as; so, this is the logistic model of microbial growth. So, even in logistic model my dear students we note that at least if N 0 is quite smaller than the carrying capacity.

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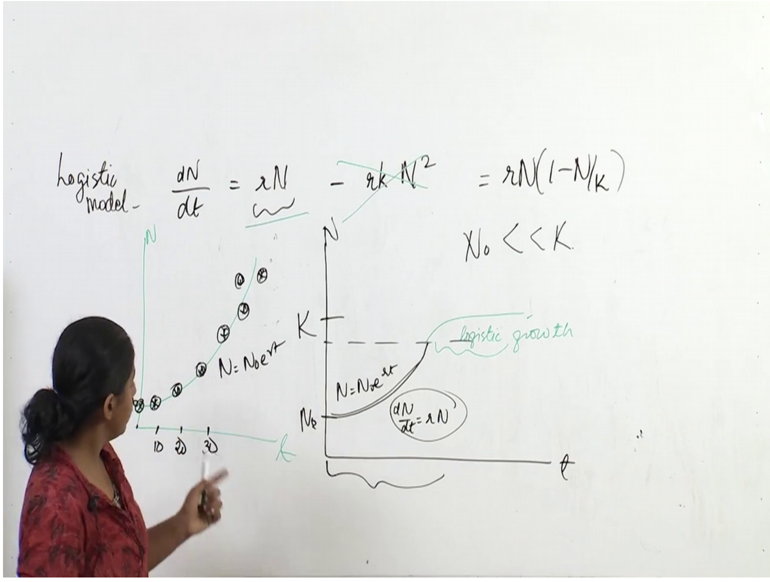

So, let us say this is carrying capacity k and a N 0 is much smaller. So, you can write it as N 0 is much smaller than carrying capacity it does not have to be very very small, but it is small enough then at least for some quite good duration, we will see an exponential growth or something that looks like an exponential growth and thus our first model which was an exponential model will be quite correct.

So, for first sometime; in this case, we will see our first model d N by dt is equal to r N holding very true and thus the population would look like N is equal to N 0 e to power rt, which is an exponential growth after it crosses a certain threshold and for this course we do not need to know, but if you are interested I highly encourage you to go and find out what the threshold would be after the threshold it will start looking like an inverted u and then it will plot u at k and this is where we call it the logistic portion of the curve, where it will the where the decay portion will become more dominant and this will be definitely a logistic growth.

So, to model this we need a logistic model to model this we can still use our exponential model and this is very important because when we grow microbes in cell in lab we usually do not have very high population. We usually prepare a very clean media that is devoid of any contamination especially microbial contamination, we put the plenty of resources and a lot of food just the right environment, if it is an Arabic micro we put oxygen in it like it is well aerated, if it is a microorganism that likes to live in less oxygen spaces we reduced the oxygen just perfect environment for the microbe.

So, resources are not a limitation the initial in ocular even though it has healthy microbes insufficient population to kick start the growth, but it is not a lot compared to the carrying capacity and thus in laboratory for quite some time we actually see an exponential growth. And so, if you come to my lab here and try to model the observations you will notice that you will have a growth like this, N by t a very exponential growth.

Now, if I ask you, but when you do the experiment you want to get a line because you cannot take data for how many microbes are present at every given instance. In fact, we cannot gather data for every given instance to begin within most microbial acids, but what we can do is we can get data point. So, it would look like this you know for starting you have a data point here, you know you started with N 0 microbes and then you know that after 10 minutes I had this many microbes, and then after 20 total minutes I had these many microbes and then after half an hour these many and so on and so forth.

So, notice here some of your data points will fall exactly on the ideal model some of them will fall up and down which is expected totally expected and it could be because of the error in our reading it could be just natural variation of microbial growth or N number of factors we really cannot understand completely, but more or less it will fall in an exponential curve now if I asked you my dear students at this data point you know what the population was tell me what is the growth rate, what is the value of r and; obviously, at this stage assume that it is exponential growth.

So, you will completely denounce the decay part and you will only focus on r N. Now how can you use this data to find out value of r now here is the thing we know the model n is equal to N 0 ert e to the power rt.

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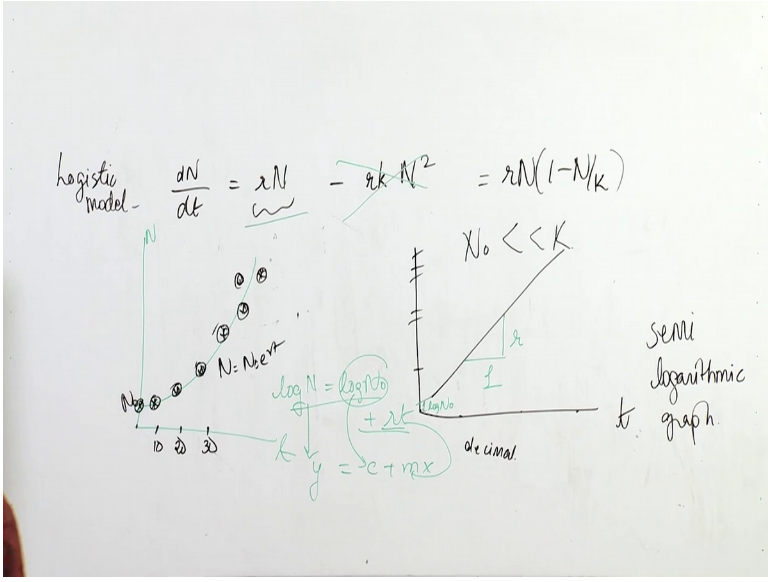

And you have so many data points and I have had students who just plug in the values of any 2 data point that they think look really good and they are sure of and then use that to calculate r. This is not a right way to go because your r might differ depending on which data points you choose one very easy and very convenient method of finding out r is to actually plot the same graph on a semi logarithmic scale semi logarithmic graph.

So, fancy way of saying that in this particular graph your x axis follows the decimal system. So, we have 0 10 20 20 and so on and your y axis has a log scale now log scale is very hard to draw by hand at least for me. So, I will not draw it, but I will show you a slide and I can tell you what looks like an exponential curve will very soon look like a straight line and why does that happen let us take a look here. So, here we have our model N is equal to N 0 by ert.

Now, time is still in the same day old decimal system, but N is now represented as in logarithmic scale. So, now, we have log N is equal to log N 0 plus rt. So, we will basically just take log of this model and; obviously, this is a straight line this is the intercept. So, this is log N 0 this slope of this line should be r because if we compare it to y is equal to c plus mx model of line then this is y log n naught is constant and rt is mx where t is x r is m m is the slope of line.

So, if we plot it in a semi logarithmic graph we can get a straight line and very quickly we can find the slope of this line and this is very easy you can do it on excel and you can also do it manually by giving a best fit line usually our vision is very good at linear analysis seeing straight line and simple patterns. So, this is much easier to do. So, here we have a population growth and some data that is observed in lab and you can notice that even though we notice quite a straight line by the way this is the semi logarithmic graph.

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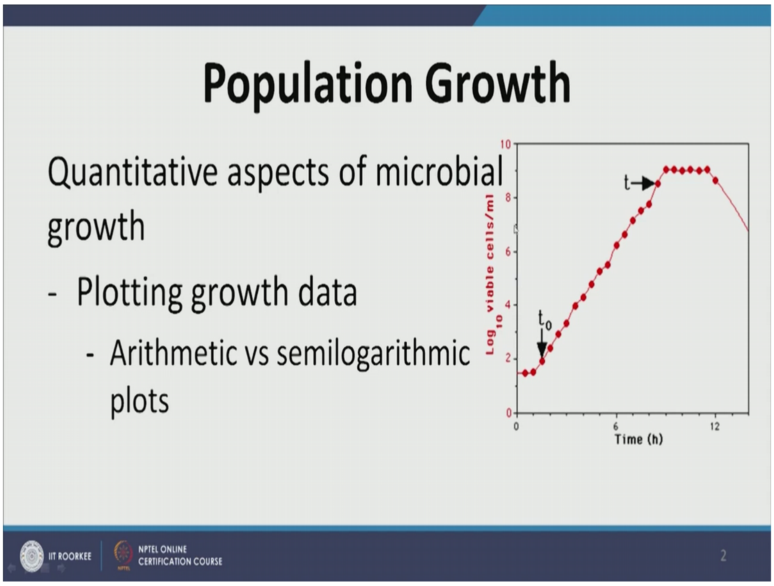

So, y axis is log of number of cells per ml and x axis is time and after few after one data point of minimal growth, we notice that we have a linear increase in population and even though it is not a perfect line you can very easily fit the best line and find the r.

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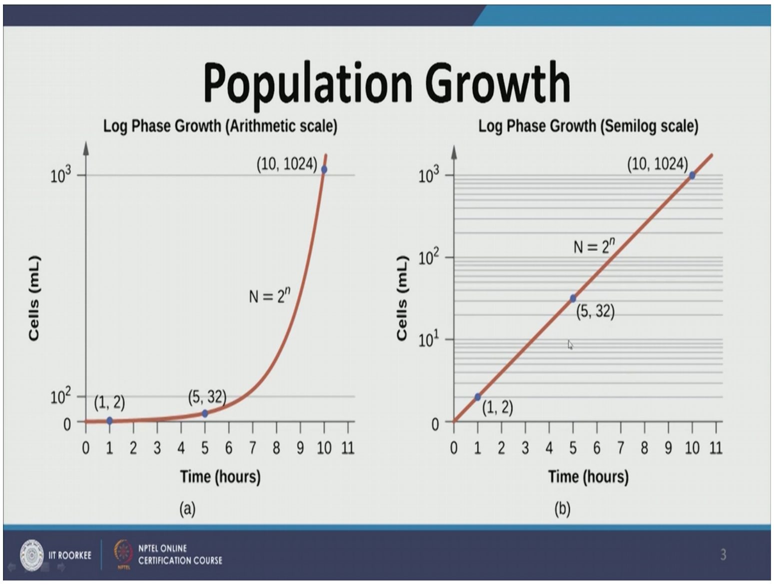

So, this is an example of what I was trying to draw and show you that on left side of this slide we have growth, which is exponential we plot the same data remember same data not new data on a semi logarithmic scale and we have a line and we can very easy find easily find out the rate of microbial growth.

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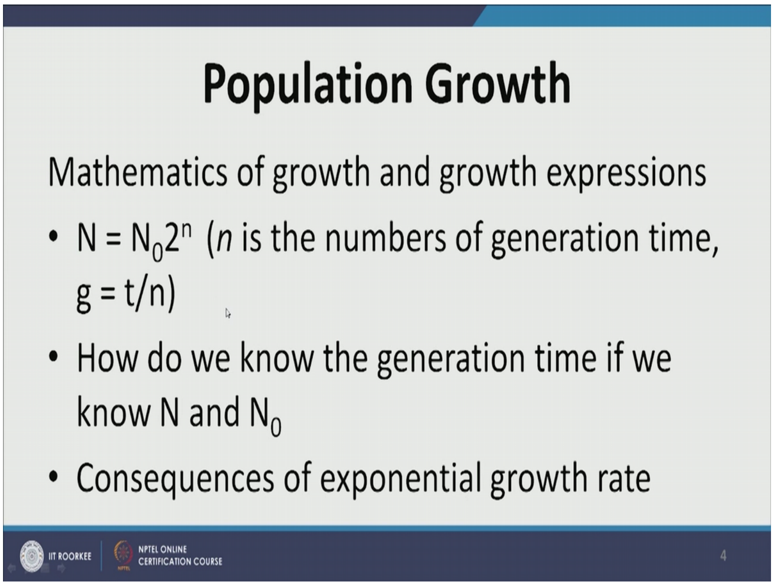

And this is something I already discussed in the previous lecture that it grows in this way and is equal to n naught 2 to power N where N is number of generations.

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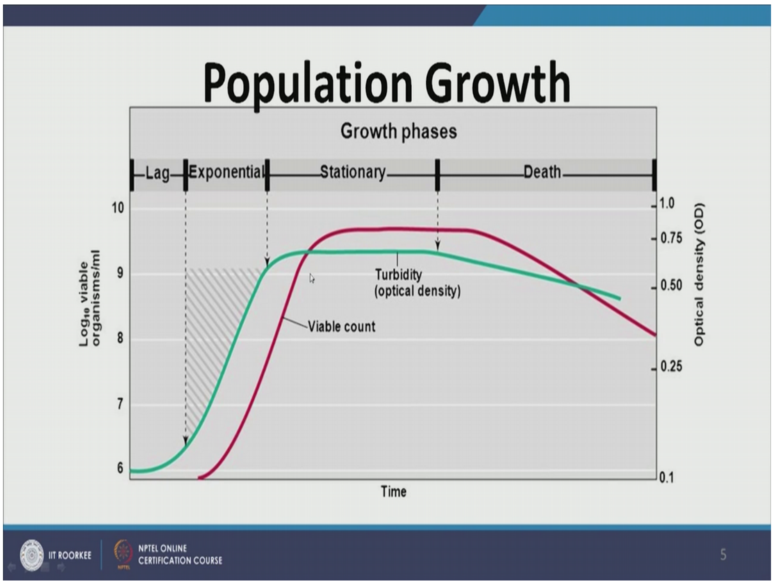

And now this is Very important we notice that in microbes initially they have a small lag phase and if you go back to the first diagram that I showed you today there is a small lag the first 2 data points have minimal growth. So, initially it is referred to as lag phase where microbes are very sluggish they do not they are still trying to settle and warm up to the media and get used to the environment and then they follow an exponential growth, which is modeled by the exponential model that we just showed you and followed by a logistic curve and then it plateaus and it stays. So, in it is carrying capacity for quite some time before it starts dying.

And how do we know this population curve how do we observe it easily in lab we measure turbidity. So, this green line actually depicts turbidity and that is what we measure and we assume that number of cells that you actually alive and kicking can be represented by the red line. So, red line is actually what you are noticing, because this is log scale it appears that this is 0, but this is not 0 it is just very small starting quantity. So, for quite some time we have a lag in turbidity increase that is a lag in micro population growth and then it exponentially rises go undergoes our logistic slowdown and then stays stationary for quite some time representing the stationary phase and then it starts decaying and dying.

So, this is how population growth looks on a community level. Now this is very important to understand that this is a community level dynamics not individual microbes dynamics. So, an individual microbe will not stay stable and not growing for some time and then have an exponential growth followed by logistics followed by stationary followed by death that is not how microbes grow, but that is how populations behave in lab. So, let us look into these each phases more in detail.

So, first phase is black phase now why do we have this phase lag phase where microbes take time to even start their exponential growth, usually the microbes that we are ready inoculate in our media they are from a different media they have been kept in a different environment they might have different temperature and they sort of undergo in a shock at times and at times they require an exclamation time which is the time they require to get acclimated to the new environment.

Now, remember microbes are quite finicky they can be quite sensitive to the environment. So, everything makes a lot of difference. So, in lag phase it definitely depends on the history of inoculum.

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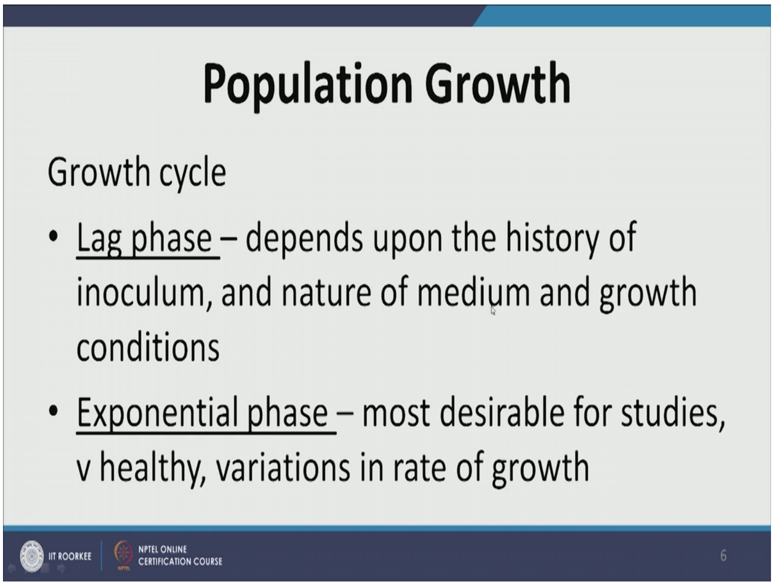

Where it is coming from what kind of media was it in and what were it is growth condition thus if they know let us say the inoculum is coming from a very rich media. So, that is they have a lot of nutrition a lot of carbon source there is a lot of sugars to eat and grow, but now I have put them in less rich medium. So, here they have less food. So, initially they will be short oh my god where is the food. So, they would not kick in an exponential growth.

It is also possible that the medium was at different temperature different period and that will further increase the lag phase now growth condition is very important now let us go back here. So, let us say I have 2 beakers and in one beaker which is beaker 1

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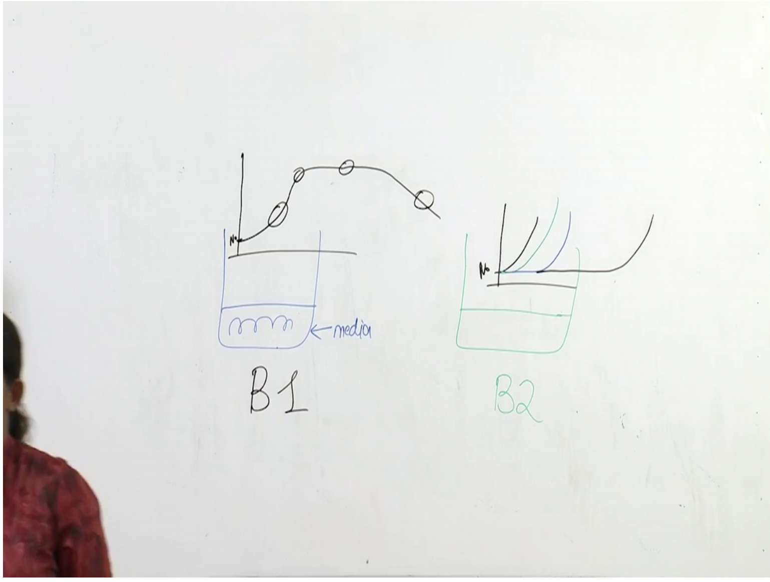

I have some media here which is in other words very tasty food for microbes and I have some microbes that are growing here very happily. So, this I can refer to as beaker one in another beaker; beaker 2 I have some media, but there are no microbes growing in it.

So, microbes in beaker one will follow the model that I just shared with you. So, their growth will look like this; initially when I first started growth in beaker one they had N 0 number of microbes the underwent an exponential growth followed by logistic stabilization they stayed in stationary phase for quite some time before decaying and dying. So, this is their growth rate. Now if I take my a sample from here and inoculate it in beaker 2 the microbes will take in the similar model here.

So, let us say I take microbes from when they are in this phase when they are in their exponential growth phase. So, microbes are already they have a momentum of growth they are already expressing the proteins required for replication of DNA required for septum and growth of body so that they can undergo binary fission. So, everything is already ready they have a nice momentum of growth and what we will notice is that these microbes when they are put here they will have a smaller lag phase.

So, in this when I add n naught they will have a very small lag phase and they will directly start exponential growth and followed by logistic in so on and so forth, but let us say I do not do this instead I take microbes when we are at the logistic curve. So, when this microbial community is undergoing a logistic phase. So, they are saying we are reaching a carrying capacity. So, they are slowing down and now they do not have as many proteins and to express, so that for replication. So, things are quite slow.

So, now in this case I will notice that they will have a longer lag phase. So, they will wait for a while and then they will shoot up in exponential growth, but let us say I take it from stationary phase. So, when microbes have already reached a carrying capacity they are quite stationary they say we do not need to grow, which is need to survive and I am just enough growth to counterbalance the death rate and then I put them here they are not very interested in growing. So, they will be like let us not grow, but after a while they will realize our numbers are much lower in beaker 2 and we have a lot of food. So, let us grow.

So, they will take time to do this and no wonder they will have a longer lag phase followed by exponential growth. Now let us say I take microbes when it is in it is decay phase. So, in decay phase they have consumed lot of resources they have produced a lot of let us say byproducts that might be some of them might be poisonous, they have run out of their fuel now and that is the beginning to die the pasture stationary phase and this is a very different model at this stage they will have and now. So, microbes are not in the prime health the microbial community and they will take a very long and they might hit an exponential curve.

Thus the lag phase depends on the environment that the microbes the inoculum was in how similar it is it also depends on how rich it is let us say it is very rich and this is not so rich. So, the lag phase will be long it also depends on where in the growth cycles this microbial community was after lag phase we have exponential phase now exponential phase is most desirable for studies if I want to study something, then I want to capture microbes that are in their exponential growth because they are they will have very small lag lag phase and they also very healthy and robust and this is ok.

And after exponential phase we have stationary phase this is where the growth has arrested, but the metabolism continues. So, even though stationary phase shows that there is no net increase in number of total cells, but their catabolism and anabolism which is basically breaking down food product breaking down things to create energy and then storing energy creating mass. So, both of these factors continue death may occur some cells might die but no overall decay.

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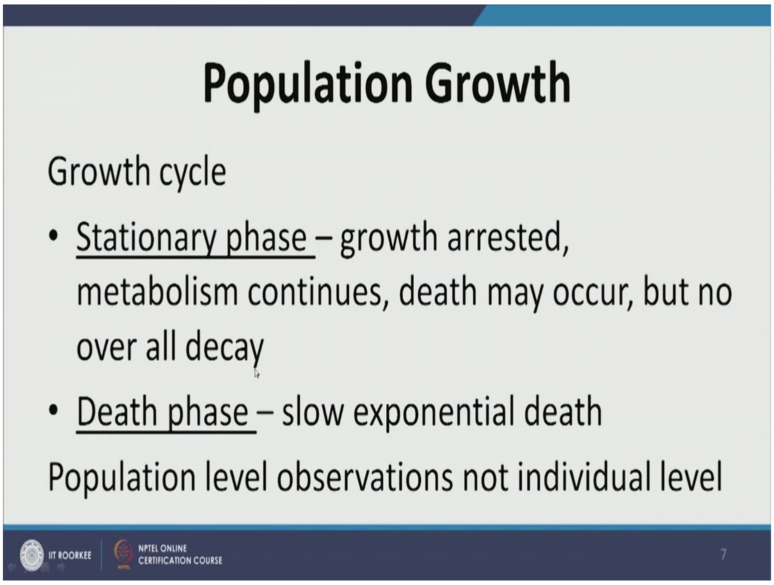

So, these are difference between death and decay death is a negative term in logistic model decay is when the d N by dt becomes negative and then they enter death phase which is a slow exponential death.

So, eventually they die, but their rate is pretty slow. So, what I drew and on the board was a straight line and then actually they undergo an exponential decay, but it is really slow and this is very important to remember that these are population level observations and not individual level observations.

So, dear students today we studied about population growth and the model that we talked about a new model logistic model and also about different phases when microbial community undergoes first growth then a stationary fail and then it dies. And we also studied about different factors that impact how the community will grow and eventually die and this is all for today. In next lecture we will jump in into microbial energetics and the chemistry of microbial communities.

Thank you very much.