Generating Engineering: Theory & Application
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Module II Basics of Biological System
Lecture 6
Microbial Growth Kinetics

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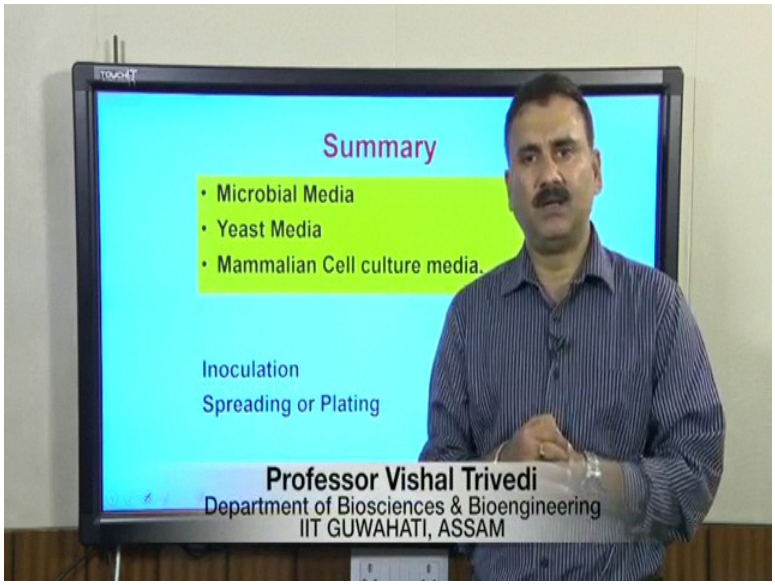

Hello everybody, this is Doctor Vishal Trivedi from Department of biosciences and bioengineering, IIT Guwahati and what we were discussing, we were discussing about the different property of the host which is actually either could be a prokaryotic in nature or eukaryotic in nature, in continuing to the same discussion we have also discussed about the different types of media compositions, what we can use for culturing these host strengths.

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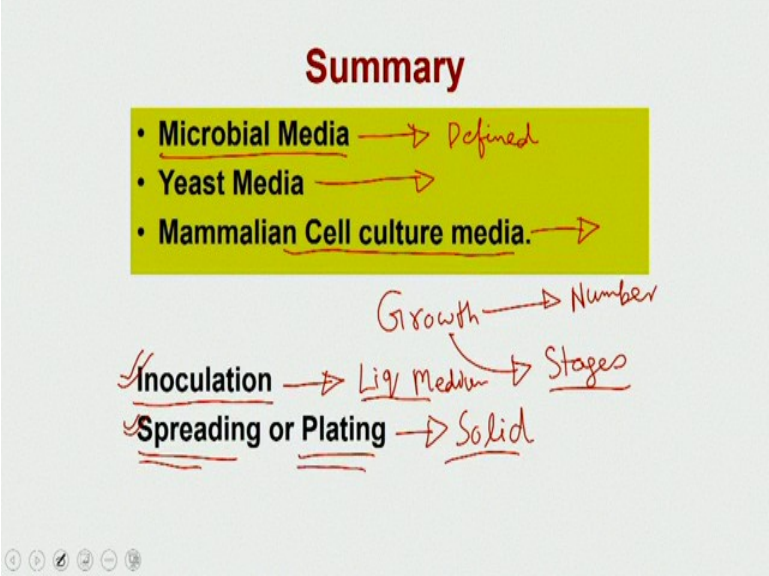

So what we have discussed? We have discussed about the microbial media and in the microbial media we have discussed about the defined media or the standard media, which is like defined media, in the defined media what we have discussed? We have discussed about the M9 and the M9 like media, where the salt and the other compositions are already known and there is no complex mixture what you are using, whereas in the case of the other kind of media, such as the LB media, you have the different components, which are actually having the mixture or different extracts and because of that it cannot be fine-tuned or controlled.

Similarly, we have discussed about the yeast media, the media what you can use to propagate the yeast and we have also discussed about the mammalian cell culture media and in this previous (chap) lecture what we have also discussed or what we have shown you is how to prepare the microbiology media as well as the mammalian cell culture media.

So the purpose of discussing this media is, because you want to grow the host cells and you want to grow the host cells so that their number will grow, and they also should go through with the different stages of their life cycle or the cell cycle and because for the biotechnology related production sometime the molecules or sometime the organisms are overexpressing or giving the particular type of (bio) biotechnologically relevant products at a particular stage of the host or sometime what happens is the particular stage of the host is important for receiving the transforming agents such as the plasmates and that is why it is important to discuss the how to study the growth of the microbial or the mammalian system.

So when we say about the growth of the microbial system, we will talk about the growth in terms of two ways, so growth can be of two types, one in which the bacteria is increasing the number, so growth could be that, where the bacteria is increasing its number or growth is, where the bacteria is going through with the different stages of the cell cycle or the life cycle.

So if you would like to grow the bacteria, you have the two options, one in which you can do a inoculation of the bacteria into the liquid media, so the inoculation you can do in the liquid media or you can do the spreading or the plating which is actually in the solid media, so depending on the kind of bacteria what you are growing or whatever is preferable conditions, you can use the inoculation techniques, to inoculate the bacteria into a liquid media or you can use the spreading or the plating techniques to plat the bacterial colonies on a solid media.

In some cases, both are these techniques are being used for different purposes, for example when you do the inoculation in the liquid media, you would like to grow the bacteria in a very large number, whereas sometimes when you would like to isolate a particular type of bacteria, then what you do is? You take the mixture of that particular bacteria and spread it onto a solid media and if you remember in our previous lecture we have discussed that if you would like to make a solid media, you can add the gelling agent such as the agar in the media and that will give you the solid media, and as you make the solid media the bacteria will grow on that solid media and you can be able to isolate a particular type of bacteria, using this techniques, so there are different modes or different methods by which the bacterial cells grow and increase their number.

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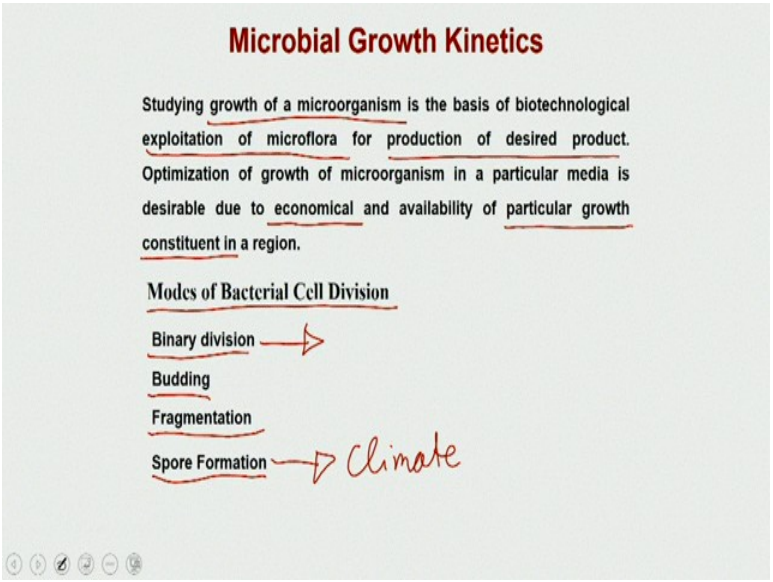

So let us see what are the different methods are available for bacteria to grow. So there are different modes of bacteria for cell division, one is called binary division, the binary division, where the cell is going to divide into two, and that is how the one, if you start with the one bacteria, it will become 2, 4, 8 and so on with every binary division.

And then you have the budding, where a small part of the bacterial cell is going to be pinched op along with the genetic material and that is how it is going to do the budding, then in the fragmentation part, what you are going to do is? The fragmentation is for the all those bacteria which are filamentous in nature, so their filamentous body is getting broken down into multiple pieces and all these multiple pieces are again re-growing and giving the individuals cells.

And at the end spore formation, so in the spore formation is actually a condition which is very much willing to the adverse climate and what happen is that if the climate is not very suitable, the bacteria is preferring to make the spores instead of increasing its number and once the climate is favourable, then this spores are re-growing and giving you, giving the new individuals.

So let us discuss how these different cell division occurs in the bacterial cell, but before that, as I discuss also that the studying the growth of a microorganism is important because it allows you to exploit the microflora for the production of desire product, as a discussed there are bacteria which actually produces the (some) products only when they attained a particular phase of their life cycle or when they attained a particular stage in their developmental stage, similarly, this is also important because of the economical as well as, (the) if you want to study the effect of a particular growth constituents in onto the production of the metabolites or the production of that (meta) biotechnology related product from those microbial sources.

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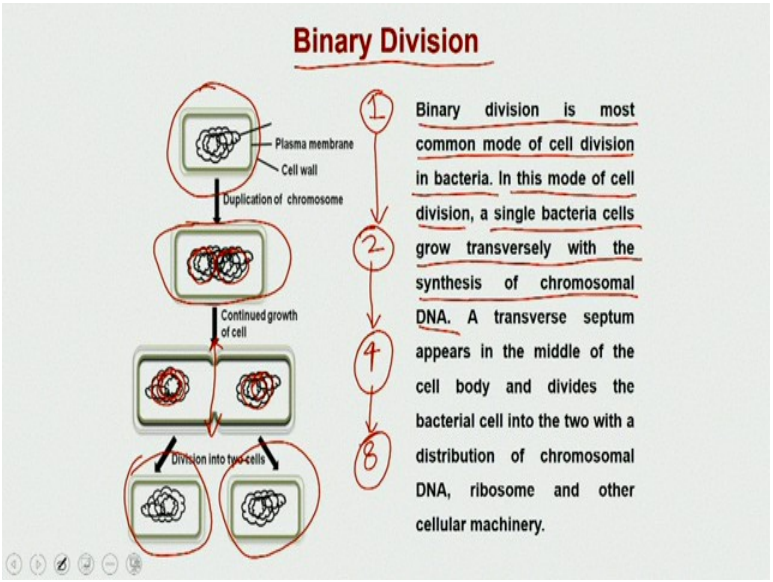

So let us discuss what are the different modes of bacterial cell division? So let us start with the binary division, as we discussed, binary division is the most common type of cell division in bacteria, in this division what happens is that a one cell in this mode, the cell division, a single bacteria cells grow transversely with the synthesis of the chromosomal DNA.

So what will happen is that single cell, which is actually having the chromosomal DNA the replicates in the transversely and once it is replicates, it also doubles genomic contents what you can see, you have one genome like this, you have another genome like this and then once it grow the genome, then you are going to have two copies of the genomic DNA, one is in this cell, the other one is in this part and then there is a transverse furrow, which actually divides this particular bacteria into the two cells, so you got, from the one single mother cell you are going to get D2 daughter cells one is this and other one is this, and that is how it actually at the end of the binary fission the one bacteria is going to get converted into 2 bacteria and if you continue like this, the two bacteria will continue to give you 4 and so on.

So actually the binary division is going to give you the duplication of bacterial cells as a number of cycle will continue and once you have the daughter cells, this daughter cells will again grow and then they will synthesise the proteins and other biomolecules which they need to run the metabolism.

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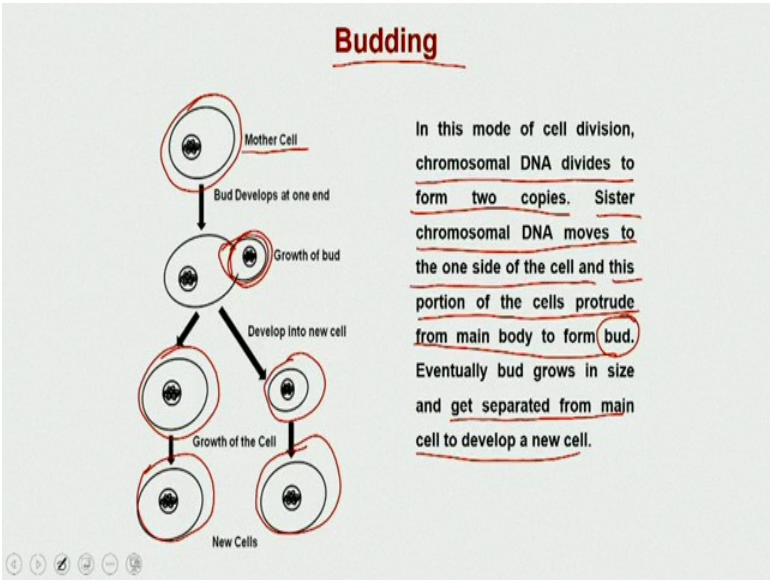

Let us move on to the next one, so next one is called as the budding, as the name suggest in the budding you have a mother cell, which is actually the parent cells or which is the starting material, in the budding what you have is the genome is going to replicate and then it get into one corner of the cell and that corner of the cell get buds out into a form of a bud and eventually what happen is this bud get pinched off from the main cell, and that is how it actually generates a daughter cell, as well as the mother cell remains like that and then the daughter cell again grow and give you the two new cells.

So the, at the end of the binary fission budding also, you are also going to see that the one cell, which is the mother cell is getting converted into two cell, so as I said, you know, in this mode of cell division, the chromosomal DNA device to form two copies, Sister chromosomal DNA moves to one side of the cell and this portion of the cell protrude from the main body to form the bud and eventually the bud grows in size and gets separated from the main cell in the form of a new cell.

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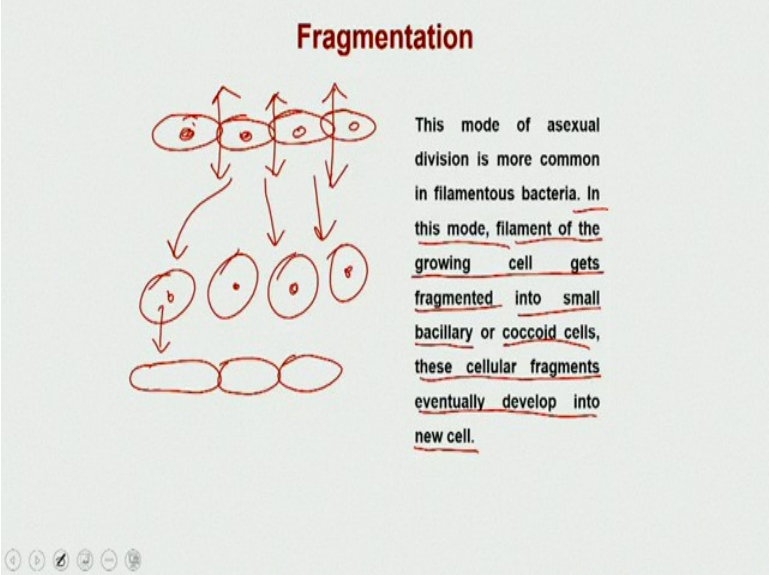

Now fragmentations, so as I said in the beginning that the fragmentation is very specific to few class of bacteria which are filamentous bacteria and these filamentous bacteria, there filamentous body is getting broken down into multiple pieces and these multiple pieces are then growing into individual cells, so you can imagine that you have a bacteria which is going like this, in the form of filament and then what happen is, this filament is going to be broken down at multiple places.

So every filament is having the nucleus in the centre and then what will happen is all these are going to form the individual bodies and that is how the filamentous bacteria is going to form the individual bodies, so all these fragments are going to grow and give the individual cells again, these cells will again grow in the form of filaments and they will give the new bacteria, as a given in this mode of the bacterial deplication, filament of the growing cell gets fragmented into small bacillary or coccoid cells, these cellular fragments eventually develop into new the cell.

So these are the few selected, these are the modes in which, through which the bacterial cell can divide and replicates into the given media, but the bacteria can, but the question comes if they are growing in number and how you are, how you could be able to monitor the growth of bacteria in a given media, so what I am trying to say is, suppose you started with one bacteria, then eventually it will form two bacteria and the two bacteria will form the four bacterias, like, so how you can monitor this, that you are whatever the media you are using is

actually supporting the growth of the bacterial cells and giving you are increasing number, so what are the matters are available for monitoring the growth of bacteria in a media.

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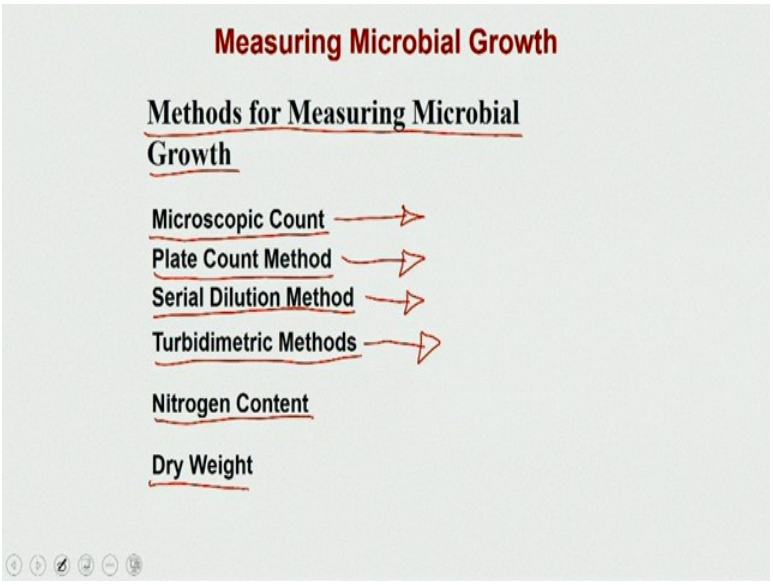

So you have the different methods for measuring the microbial growth, what are these methods? You have the microscopic methods, where you can use a microscope and you can use a microscopic chamber to count the number of bacteria in a given particular area, then you have plate counting method, in plate counting method what you can do is you can plate the cells or you can plate the bacteria a plate and then you can count the number of colonies what you are getting in a plate and that actually indirectly, you can use the convergent factor to calculate the number of bacteria which are present in your given solution.

Then you can also use the serial dilution method and in the serialisation method what you can do is, you can dilutive the bacterial colonies in liquid media and then eventually you can use the plate counting method to count the number of bacteria present in your diluted media, you can also use the turbidimetric method, turbidimetric method actually utilises the physical properties of a bacteria, so you know the bacteria is actually going in a media and it is giving, it is scattering the cells, so that actually provides the turbidity in the media and that turbidity the can be measured using the different, using the spectrophotometer and that can be correlated with the number of bacteria which are present in a given media.

Then you can also use the nitrogen content or the dry or the fresh weight content as well to measure the number of bacteria which are present in a given media, or given solution, so let us start with the microscopic count.

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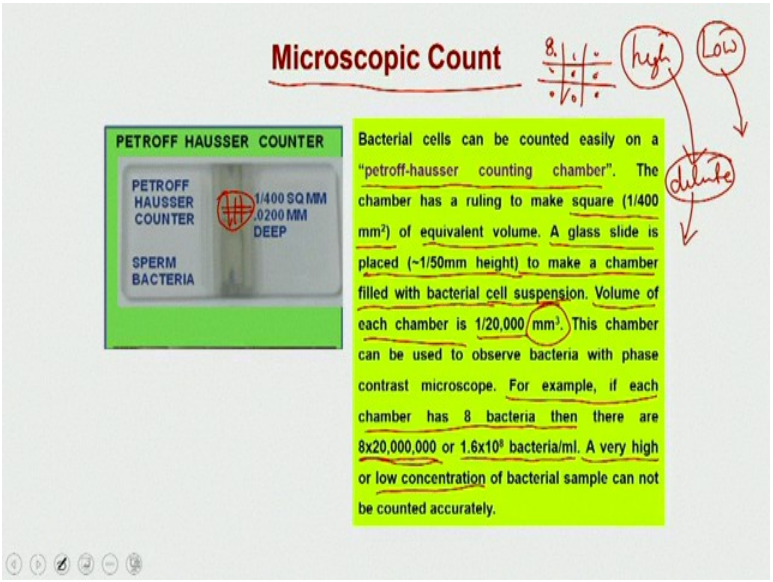

So in a microscopic count what you need is? You need a cell counter and this is a specific cell counter which is called as a petroff-hausser counting chamber, what you have is? You have a chamber in the centre and where you can actually put the cells and these counter, these chambers have a square which is actually of a size of 1 raise to 400 millimetres square and it gives the equivalent volume, so what you have to do is, you have to put the bacteria in this particle chamber and on top of that you have to put a glass slide, so that they will be evenly distributed into this chamber and the bacterial suspension will be filled into this chamber and then what you do is and as you can see the volume of this chamber is a approximately 1 raise to 20,000 mm cube.

So if you count the number of bacteria using a phase contrast microscope, because the bacterial cells are going to reflect the cells, reflect the light and because of that you can actually be able to use the phase contrast microscope to see the bacterial cells, so you cannot be able to see the bacterial cells, what you can see is a dust like particles into the cover slip and this is just like particles are going to be moving, so that is how actually it will say that these are not dust, but the bacterial cells.

And for example, so when you count the number of cells? How you are going to calculate the number of bacteria which are presents, so for example if you, if each chamber has 8 bacteria, for example, so it has actually a chamber like this, so what you have to do is? You have to count in each of these chambers the number of bacteria present. Okay, so if you count the number of bacteria and suppose in this, you have the 8 number of bacteria, then multiply that

number to this number, which is actually a convergent factor or the number of bacteria which is, if you want to convert it into to the number of bacteria per ml and the number what you are going to get is 1.68 into 10 to the power 8 bacteria.

So if you have a very high number or a very low number because if you count very little or if you count very high, there is a chance that your counting may not be correct, so in those cases what you have to do is? If it is a very high number, you might have to dilutive the bacteria and you can do a serial dilution and then you can do this diluted solution, you can use for counting the media, if it is a very low number, then what you have to do is? You have to concentrate this number and you have to use the concentrated number and you have to use the more volume, so that the number of bacteria present in each chamber should be optimal, so that the your counting should be statistical significant and you should make the less number of errors.

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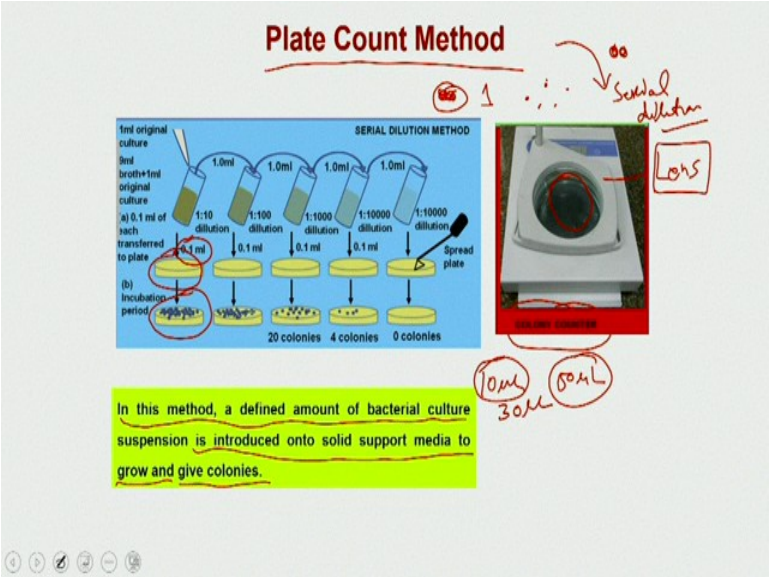

Let us move onto the next, so in the next what you have is a plate count method? In the plate counting method what you need is? You need a agar plate, so that where you are going to plate the bacterial cells or where you are going to plate the bacteria which is present in the liquid culture and then also what you need is the colony counter or the instrument what is the called as colony counter, so let us discussed first the method and then we will discuss about the colony counter.

So in this method what you have is defined volume of bacterial culture, it could be 10 µL, 30 µL, it could be 50 µL, so there is no hard and fast rule that you cannot take anyone you like,

depends on the two condition that is, the amount of bacteria what you plate should give you the short fiction number of colonies, so that the when you do a accounting, it should be statically significant, so if it is too low, you can actually go for the higher volumes, in it, it is too high, then you can go either to a lower number or you can do a serial dilution of the concentrated stock and you can do the counting using the colony counter.

Because it is giving too many colonies, then it is possible that the two colonies, you have two colonies very nearby particular count this as one colony and in other cases it could be possible that you only have couple of colonies and this colonies may not be statistically significant to give you the real number.

So what you have to do is? You protect a defined amount of bacterial culture and then you plate those cultures into a solid support media, so that the bacteria will grow and they will give you the colonies, as it like we have discussed here, so what you do is you take the 100 µL of bacteria put it onto the plate and then let them grow for another 16 hours and that actually will give you the individual colonies and then what you do is?

You take this plate and put it into the colony counter and colony counter is actually a instrument where you have a place to keep the plate and below you have a light source and on the top you have a lens, so it actually having a magnifying lens, so because of that could be able to see the two colonies, even if they are very close by, you could be able to see them separately, so with the help of the lens you could be able to count each and every individual colonies.

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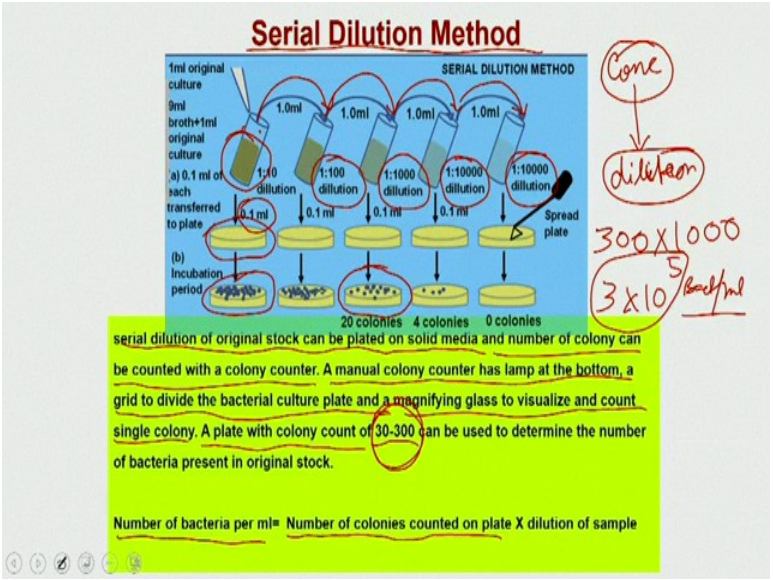

Now let us move onto the next method, so next method is called as a serial dilution method, so in the serial dilution method, serial dilution method is actually where you original stock will be listed on a solid media and the number of colonies can be counted with the colony counter. Okay, a manual colony counter has lamp at the bottom, a grid to provide the bacterial cultural plate and a magnifying glass to visualise and count a single colonies. Okay, in this particular type of colony counter, a call a number of 30 to 300 can be used to determine the number of bacteria present in a original stock.

So what happen in a plate counting method? When you have a very concentrated stock of bacteria, the number of colonies what you get is beyond this number, means you are getting thousands of colonies and the other problem is that the two colonies are merging to each other and that is how you are could not able to distinguish whether it is a single colony or it is a double colony, so in those cases, this concentrated stock has to be the, you have to dilute and this dilution you have to do in a serial dilution methods.

So in a single deletion method what you have to do is first you take a original stock, for example in this case we have taken a 100 μL and added it to the 1ml, so that actually is 1 raise to 10 dilution from this, what we have done is we have taken a 1ml and then it, we have again added to the another 1ml and then finally you can take from here and make another dilution that will give you 1 raise to 1000 dilution and then from here you can make another dilution that will give you 1 raise to 10,000 dilution and so on and from then ultimately what you do is you take the 100 μL of the each ml and from each of this culture, which is diluted of 1 raise to 10, 1 raise to 100, 1 raise to 1000 and 1 raise to 10,000 or 1 raise to 100,000 and then you plated onto the cell.

Okay, plated onto the agar plate and what will happen is all this will give you the colonies, which you can count, so this will give you the number of colonies, so what you can do is once the number comes in this range, you can take that particular plate and then how you can calculate the number of bacteria per ml, you can take this number, so the number of colonies what you are going to get and the particular plate, for example if you got the 300 colonies.

Okay and suppose you made the 1 raise to 10,000 dilutions okay, so then you can just multiply this number by the number of dilution what you have made okay, so this will become 3 into 10 to the power 5 bacteria per ml, similarly, if its, if you decrease the dilution this number will go up or if you increase the dilutions this number will go down, but this number will increase, so ultimately you can actually calculate the number of bacteria present

in 1 or 2 dilutions and that actually will give you the average number of bacteria which is present in this particular solution what you have started from the beginning.

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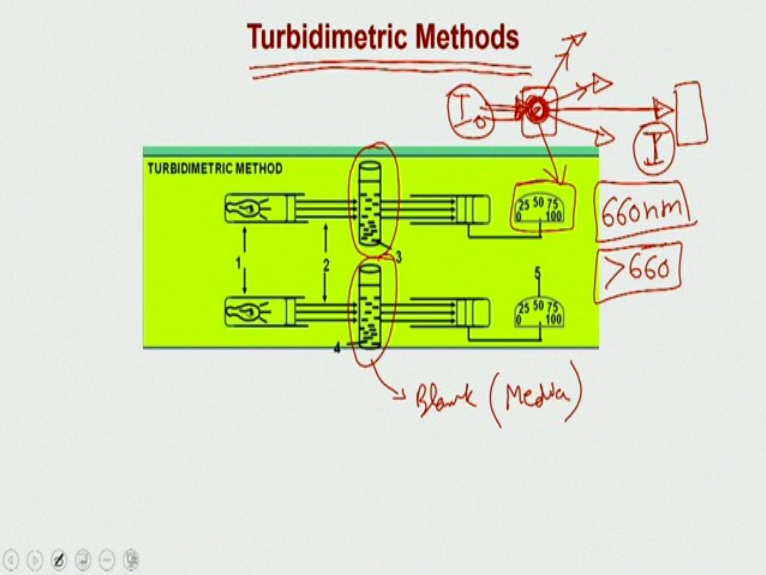

Now will move on to the another method, which is called as the turbidimetric method, so you know the bacterial cell is made up of, the bacterial cell is particulate in matter, so when the bacteria is growing into the media, it actually gives the turbidity or it actually scattered the light, so it is actually a object, so if you can imagine that this is your bacteria, so when the light will fall into this bacteria, it actually scattered the light in all the directions okay, so that actually can be used in.

So it is just like a dust particle, so because the cells are growing and the cells are live and because they are scattering the light in all the direction, there is a loss of light, there is a loss of intensity, when a particular light beam is heating the bacterial cell and it is actually getting loss because what you are measuring? You are measuring into the detector this light, whatever is coming out from the sample, but it is moving away on a particular angle which your detector cannot detect, so because of that if you are making a light I it is actually becoming the, if you heating the light with the I0, it is becoming of a light of I and that is, these differences of I versus I0 can be used to correlate the number of bacteria which are present in your solutions.

So as you can see in this system, which is actually a spectrophotometer, what you have is? You have the, what you are going to do is you take the bacteria into qubit and on the parallel you will put the blank, so in this case you will put a blank, so blank, what is a blank? Blank is

going to be your media what you using for culture in this bacteria and you put the media as a blank and then you put the bacteria as a growing bacteria into the other qubit and then you collect the absorbance or the scattering of cells at 660 nm or any wavelength, which is bigger to this number, because if you go anything beyond 660 number, you can be able to mirror this scattering of the cells and then the number what you get is actually the, you are measuring the scattering or the turbidity of the solution and that actually can be correlated to the number of bacteria which is present in the solution.

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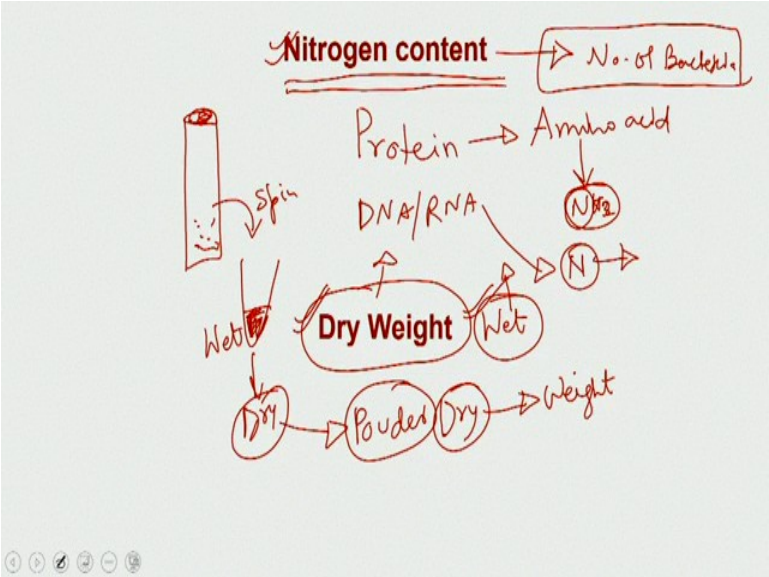

The next method is that you can use the nitrogen content, so you know that the bacterial cell is made up of different types of biomolecules and in the previous lecture what we have discussed? We have discussed that the bacterial cell is made up of the proteins, made up of carbohydrates, DNA, RNA and the lipids and the protein is made up of, so the protein is made up of amino acids and amino acid means, it contains the amino group okay, this means the nitrogen and apart from this, the DNA or the RNA also contains the nitrogen because they have the purine or pyrimidine bases and these purine and pyrimidine bases are containing the nitrogen and apart from that the some of the other biomolecules also are present, which are also containing the nitrogen.

So if you would like to count the number of bacteria in a given culture media, what you can do is, you can calculate the amount of nitrogen which is present that is actually going to be correlating to the number of bacteria which is present in the, in that particular media because the nitrogen is, the cell bound nitrogen is going to be indirectly say that these are the number of bacteria present in the solution.

Similarly, you can also do the dry weight or the wet weight, what is the difference between the wet weight and the dry weight is? Suppose you are cultural the bacteria in a vile okay and you have the number of bacteria okay, so what you can do is, you spin this bacteria and when you spin it actually will give you a plate of bacteria okay, now what you do is, so these are the wet bacteria.

Now what you do is, you dry them okay, so that actually will give you a powder, the bacterial powder and this is actually dry bacteria, so if you can gather this dry powder, you can actually weight this bacteria using the weight machine and then you can say that this many milligrams of bacteria per ml of culture volume and that is also a standard way of comparing the two solutions, so whether it is a dry method or the nitrogen content method that is not going to give you the actual number, because you do not have a reference point, but these are the methods which people used to compare the number of bacteria present in two different samples.

So suppose you are actually over expressing a particular protein or suppose you are trying to receive a particular type of metabolites okay and in that case suppose you are modulating the media compositions and trying to see if that actually going to support the bacterial growth or not, in those kind of studies you can actually use the nitrogen content method or the dry as well as the wet method okay.

So dry method is actually more superior compared to the wet method because the wet method is having a liquid part, which is actually the media part and that actually could vary from batch to batch and because of that it may give you the some kind of artefacts, compared to that the dry method is going to give you the consistent result if you do from pasta batch, so these are the different methods what people can use to monitor the growth of the particular bacteria in a culture volume or in a particular media.

Let us discuss further that how the bacteria will growing in a particular media and what are the different phases it which go through, so when a bacteria grows in a particular media, it has to go through with the different phases and then only it replicates and increase its number.

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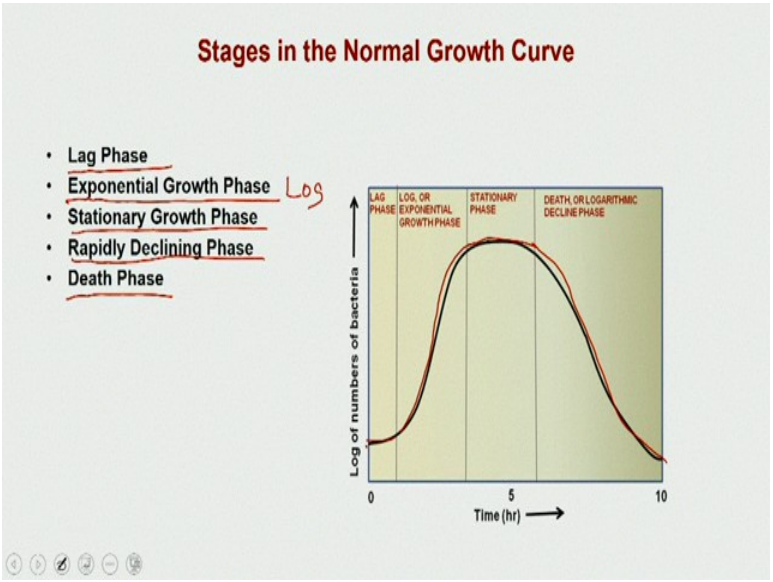

So these are the, so what are these phases? As you can see in this particular graph, one is called, the first one is called as the lag phase, the other one is called as the exponential growth phase, this is also called as the log phase, then you have the stationary growth phase, then you have the rapidly declining phase and then you have the death phase, so as you can see log of number of bacteria versus the hours and what you can see is that is has a lag phase, then it has the log phase, then it has a stationary phase and then ultimately it has a decline phase, as well as the death phase, so let us discuss these phases and the relevance of the phases as in context to the media components as well as the compositions.

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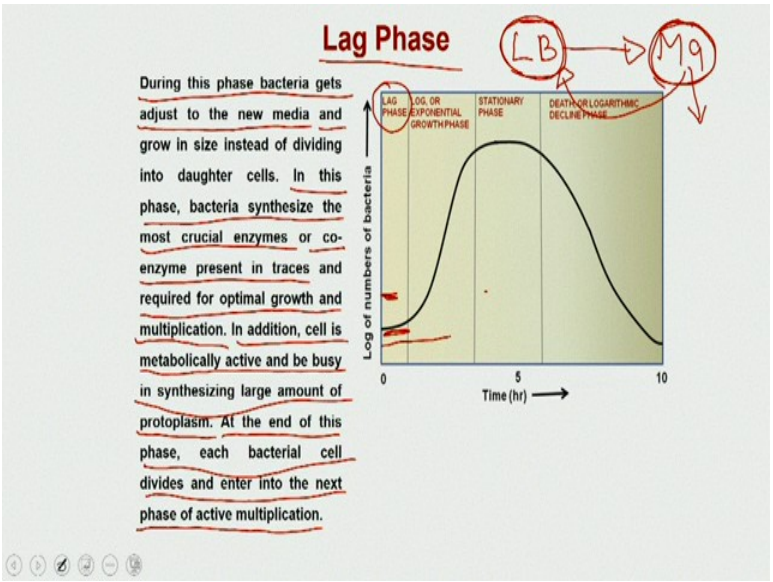

So the first is called lag phase as the name suggests, lag phase means the bacteria will, is not going to, bacteria be lag in terms of its growth, which means it is not going to grow very rapidly and doing this phase, what is the most important thing is, that bacteria get adjusted to the new media, which means if you are taking a bacteria from a rich source, or if you are suppose taking a bacteria from the LB media and if you are putting it into the M9 media and you know that the LB media is a rich media, whereas the M9 minimal media is not that rich media, the bacteria will normally been actamatized to the LB media, but it is not actamatized to the nutrient source or nutrients which are present in the M9 source.

So because of that it has to get adjusted okay, but it is not true that if you do the reversal, like for example if you are growing bacteria in a M9 media and then you add it, add those bacteria to the LB media there will be no lag phase, there will be still be lag phase because the bacteria has adopted the biochemical reactions, according to the M9 media, but now you have put it into a rich media, which is called the LB media.

So once it reach to the LB media, it may not require those biochemical reactions which it was running in the M9 media, it may maybe just down those biochemical reactions and because in M9 media, it may not be getting those particular type of biomolecules and because of that it needs to be synthesised those biomolecules when it is present in the M9 media, but once it reaches to the LB media, it does not required to run those biosynthetic pathways to synthesise those essential molecules, so as a result, it has to shut down and that actually requires the adaptation and that is how it is actually going to have a lag phase.

So lag phase is always going to be there as long as you put the bacteria from one media to another media, irrespective of that whether you put the media from rich media to define media or from the less rich media to more rich media, what happens is in this media, so once you adopt, you actually will avoid to go for dividing the cells because the division starts once only that bacteria is happy and bacteria got adjusted to the particular type of conditions.

In this particular phase what bacteria will do? Bacteria will synthesize the most crucial enzymes or the coenzyme which are present in traces, which means if the media is not providing some of those biomolecules, than it actually going to starts synthesising those molecules, then why it is doing so? Because it has to optimize the growth and is the conditions which are required for growth and multiplication, in addition, the cells are going to

be metabolically very active and it will be busy in synthesising large amount of protoplasm, which means the cell will grow in size, but it will not growing numbers.

So at the end of this phase, the is bacterial cell will divide and enter into the next phase of active multiplication, which is actually called as log phase, which means in this particular phase, the bacteria will only going to adjust to the new environment. Okay, whether it is a rich environment, or whether it is going to be a poor growth conditions, when have the rich environment the lag phase is going to be smaller, if it is a poor conditions the lag phase may be going to be higher.

So the length of lag phase actually defines whether the media compositions are actually supporting the growth or actually whether they are causing the some kind of stress to the cells because if it is more lengthy, which means some of the media components might not be supporting the growth of this particular bacteria and that is why the bacteria is taking more time for adaptations.

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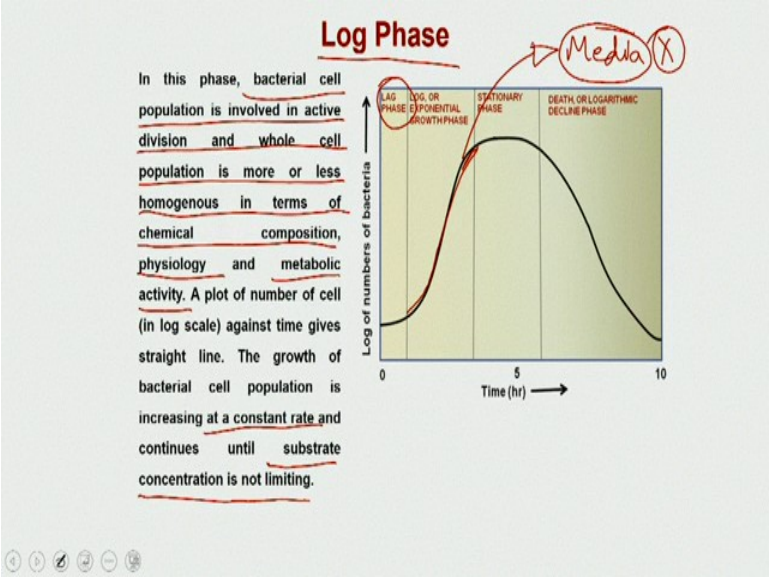

Now let us go to the log phase, so the log phase is in this phase, the bacteria population is involved in active division and the whole cell population is more or less homogeneous in terms of chemical compositions, physiology and metabolic activity, in this particular time log bacteria number of colonies versus time is actually in proportion, which means if it is, if you count the number of bacteria, the bacteria cells replicates very rapidly and that is because the bacteria cells are increasing at a constant rate and it continues.

So the log phase, how long the log phase will continue? Until the media components are providing the nutrition, so once the bacteria got adjusted into the lag phase, than it will do a rapid growth during the log phase or the exponential growth phase and it will continue until the bacteria will keep getting the substrate molecule or the nutrients from the media, once this phase is over or the bacteria will reach to the end of the log phase, then the media components are not going to give them the enough nutrients and that is how they will enter into the next phase, which is called as the stationary phase.

So in the log phase, the number of bacteria which are going to grow is more and that is why compared to the number of bacteria which are going to die, and that is why the number of bacteria are, the number is increasing in proportion to the time.

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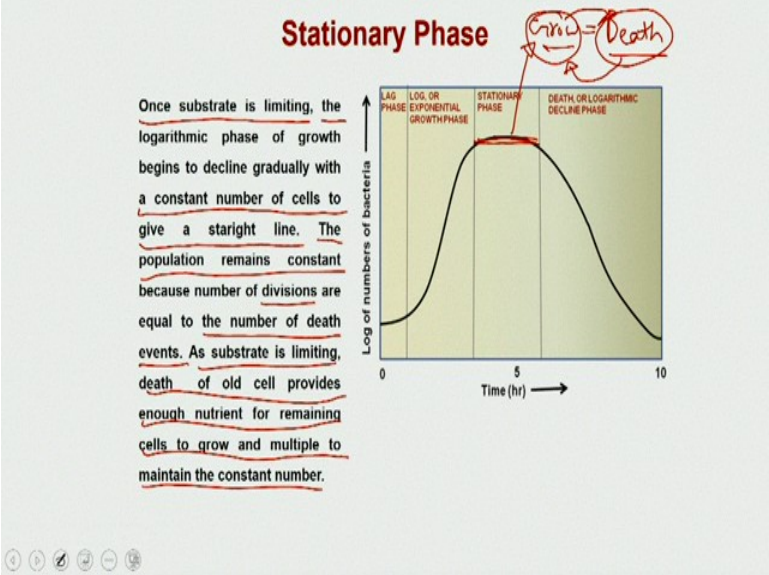

Now, once the nutrients are limiting, because once the bacteria will grow it will used up all the nutrients and that actually will use to increase the number, it is eventually going to deplete the nutrients which are present in the media and that is how the substrate is going to be limiting and then it actually enters into the stationary phase and which means it actually going to maintain the number of bacteria into the media, which means the number of bacteria.

Which will be going to grow is equal to the number of bacteria which are going to go for the death phase, which means the number of bacteria which are going to reproduce, the number of bacteria are going to die and because of that, and why that is required because the number of bacteria which are going to die, they will actually going to release the nutrients and these nutrients are going to be used by the remaining bacteria for the growth and that is how this

cycle will continue, which means the older bacteria will die and they will release the nutrients and that nutrients is going to be used by the new bacteria and that actually will give you the stationary phase, which means the number of bacteria in a colony will remain constant, that is what is written here.

The population of the bacteria colony will remain constant. Okay, because a number of division are going to be equal to the number of death events, as substrate is limiting, the death of old cell provides enough nutrients for the remaining cells to grow and multiply to maintain the constant number, now once this arrangement that, death of older bacteria will provide a nutrient for the new bacteria will not be also support the growth of these bacteria, then they will go into decline phase or the death phase.

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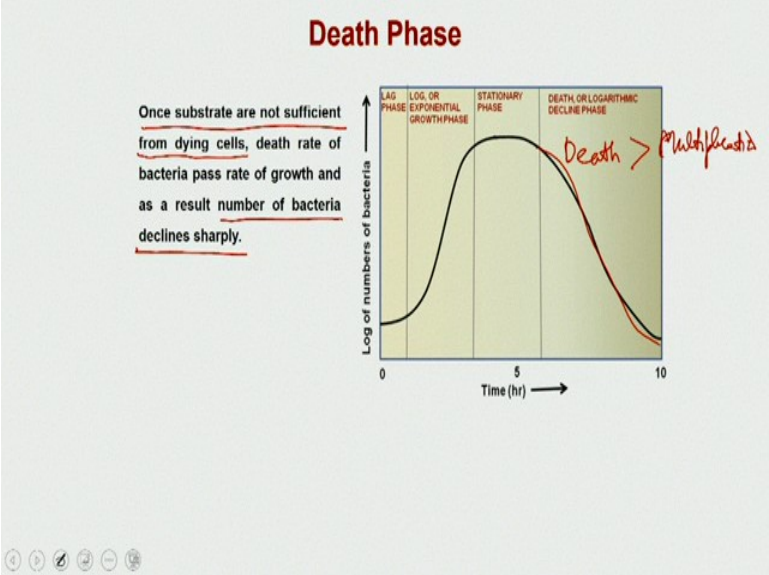

That is what is written here, once a substrates are not sufficient the cells were start dying and they will enter into death phase, so in the death phase, you are going to have, the more deaths compare to the multiplication or the division and as a result, what will happen is the number of bacteria is going to decline very sharply into the media and eventually what you see is that there is no bacteria which is growing in this particular microenvironment okay, so this is about the growth of the bacteria, in a growth of a particular bacteria in a given media.

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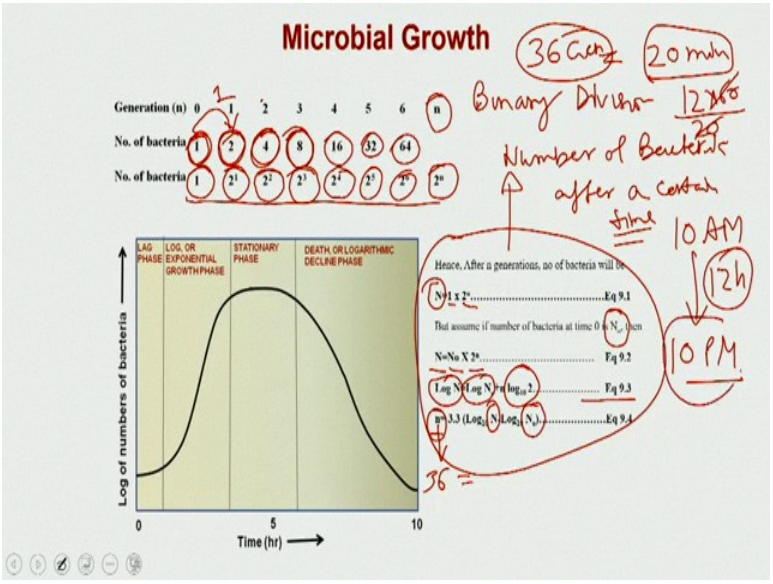

And a suppose if you summarise what will happen is that they are going through a binary phase, binary division. Okay, then what is mean by binary division is, support, we started with the one number of bacteria. Okay, then once they will complete one cycle or the one round of multiplication the bacteria will become 2, the bacteria will become 4, bacteria will become 8, another 16, 32, 64 and so on and the time in which the bacteria will take, will grow from one bacteria to two bacteria is called as the generation time or the generation number.

So in first-generation the one bacteria is going to converted into two bacteria, if you have two generations then it becomes four, if its three generation it becomes eight and if I count the number of bacteria which is present, then it will be, if it is second generation then it is going to be 2 to power 2, if it is third-generation it is 2 to power 3, that is what you see 2 to power 3 is 8 actually and if it is 4, then 2 the power 4, 2 to the power 5, 2 to the power 6.

So you can imagine, if you have to count the number of bacteria in Nth generation, than it will be 2 to the power N, which means if you take this into the equation form the number of bacteria will be 1 into 2 to the power N. Okay, so suppose you started with the number of bacteria which is N0 and you want to calculate the number of bacteria which is, which going to be after the Nth generation, then the N is equal to N0 into 2 to the power N generations, 2 to the power N and if you put the log, then it becomes log N equal to log N0 plus N log 10 2, which means if you simplify and calculate this equation, it becomes N equal to 3.3 log 10 N minus log 10 N0 and where this N is the number of generations and the N is the total number

of bacteria and N0 is the starting bacteria or the number of bacteria what do you have inoculated into the media.

What is the application of this particular kind of equation? This particular kind of equation has application that it actually allows you to calculate the number of bacteria after a certain time, for example if I have inoculated the bacteria at 10 AM in the morning and I want to know that how many bacteria would be there, then I would like to take of the media at 10 PM, which means actually it is 12 hours, so this actually I can do because if I have a bacteria which is of 20 minutes as a life-cycle okay, which means in 12 hours how many recycles? 12 into 60 divided by 20, which is actually 36 generations. Okay.

So if you put the 36 into N okay, then you can be able to calculate the number of bacteria what you are going to have at 10 PM, what we have discussed that the different times of multiplication modes, what is present in the poker attic system, we have also discuss the different type of techniques as well as the methods to measure the bacterial growth and then at the end we have also discussed about the different phases through which the bacteria cell goes through an attained, do the growth within the media,

So as we said, it is actually falling a lag phase, log phase, stationary phase as well as a death phase and most of these phases are having its own significance in terms of its application or in terms of their utility to exploit the microbial cells and so with this we would like to conclude our lecture here and in subsequent lecture what we are going to discuss? We are going to discuss about the transforming agents and now we have already discussed about the host and its metabolism as well as the other kind of parameters.

So in the subsequent lectures what we are going to discuss about how you can actually isolate a particular gene from the host, so that you can use that gene to generate the chimeric genetically modified organisms and how that gene can be manipulated to generate the novel class of transforming agents and what are the different types of transforming agents are available for our biotechnology related applications. Thank you.