19. Bacterial growth- population growth and growth cycle – continuous culture – chemostat and turbidostat; synchronous culture

Growth rate: Increase of bacterial cell number per unit time is referred as growth rate

Generation: The interval of formation of daughter cells from parental cell is called as generation

Generation time: Time required to form two daughter cells from a single cell is called as generation time. This is also called as doubling time. *E. coli* has the doubling time of 20 min and *Rhizobium* has 2 h.

Principle:

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 1) .The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.
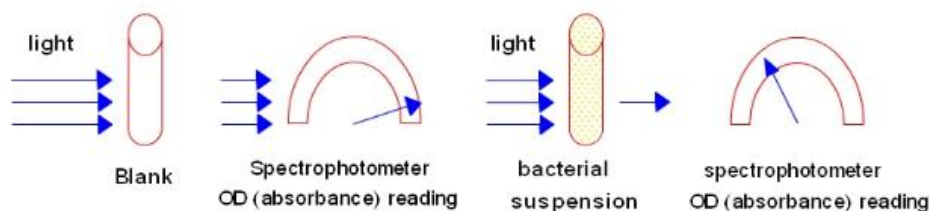

Fig 1: Absorbance reading of bacterial suspension

The growth curve has four distinct phases (Fig 2)

1. **Lag phase**

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent.

2. Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 2^0, 2^1, 2^2, 2^3.........2^n, n is the number of generations) This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms. *E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes. The metabolites produced during this phase are called primary metabolites hence it is also called as **trophophase**
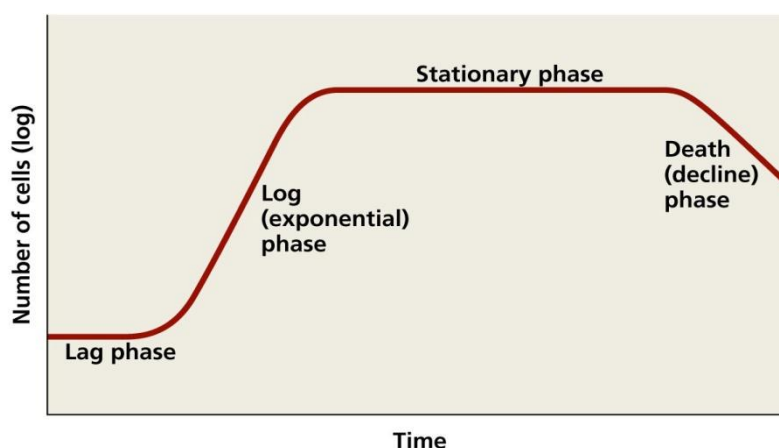
3. Stationary phase

As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This result in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual. In this phase they produce certain substances other than primary metabolites such as antibiotiocts. These are called as secondary metabolites and play no role in the growth of the microorganisms. This phase is also called as **idiophase.**

4. Decline or Death phase

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavourable

conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores.

Fig 2: Different phases of growth of a bacteria

Generation times for some common bacteria under optimal conditions of growth.

Bacterium	Generation Time (minutes)
Escherichia coli	17
Bacillus megaterium	25
Streptococcus lactis	26
Streptococcus lactis	48
Staphylococcus aureus	27-30
Lactobacillus acidophilus	66-87
Rhizobium japonicum	344-461
Mycobacterium tuberculosis	792-932
Treponema pallidum	1980

Mathematics of Growth: Microbial growth during the exponential phase is very important and of interest to microbiologists and the analysis applies to microorganisms dividing by binary fission. The time required by a cell to divide is called the generation time or doubling time. In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3 ....2^n (where n = the number of generations). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature. This might vary from organism to organism depending upon the environmental conditions etc. For example in *E.coli* the generation time is 20 min and hence after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. The population is doubling every generation; hence the increase in population is always 2^n where n is the number of generations. The resulting population increase is exponential or logarithmic.

When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. If we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. The **generation time** is the time interval required for the cells (or population) to divide.

G (generation time) = (time, in minutes or hours)/n(number of generations)

G = t/n

t = time interval in hours or minutes

B = number of bacteria at the beginning of a time interval

b = number of bacteria at the end of the time interval

n = number of generations (number of times the cell population doubles during the time interval)

b = B x 2^n (This equation is an expression of growth by binary fission)

Solve for n:

logb = logB + nlog2

n=logb-logB/log2

n= logb-logB/ .301

n = 3.3 logb/B

G = t/n

Solve for G

G= t/3.3logb/B

Example: What is the generation time of a bacterial population that increases from 10,000 cells to 10,000,000 cells in four hours of growth?

G= t/3.3logb/B

G= 240minutes/3.3log10^7 /10^4

G= 240minutes/3.3x3

G = 24 minutes

Synchronous growth of bacteria : All the cells of a bacterial culture a same stage of their growth cycle is referred as synchronous growth.

When bacterial growth which we discussed earlier as growth curve, if observed under microscope, will have different stages of its reproduction ie, cell elongation, septum formation, divided cells etc. So, The cells and cell divisions will not be uniform. But, in synchronous growth culture, the bacterial cells will be of uniform stage.

The synchronous growth of bacteria will have the growth curve as following graph :

The synchronous growth of bacteria can be achieved by growing the cells at suboptimal temperature (below the optimum temperature). E. coli needs 37°C for its normal growth and if grown at 28°C, the synchronous growth will occur. This type of culture will be useful in molecular biology especially the gene cloning and transformation studies.
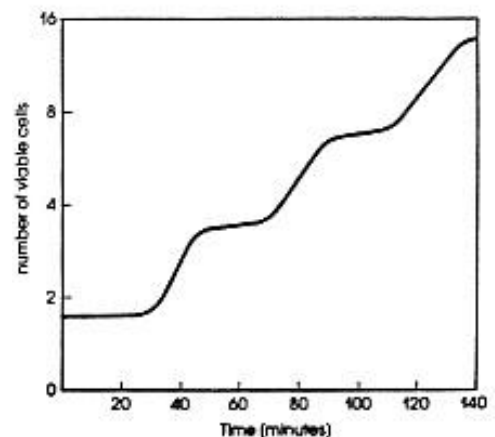
Steady state of growth of bacteria : maintaining the bacterial population in a particular stage / phase of the cycle is referred as steady state of growth.

Batch culture

The growth curve which discussed earlier is meant for the bacterial culture in a constant media referred as **batch culture**. In this type of culture, the maintenance of bacteria at particular stage is not possible.

- The "batch culture" is also known as "closed culture" system

- In this system, in the beginning, the nutrients and other additives are added in required amounts
- There is no refill of nutrients once the fermentation process has started and the product is recovered at the end of the process
- Once the process is completed, then, the fermentation vessel is cleaned properly, sterilized before it use for another batch process
- In the beginning, microorganisms grow at a rapid rate due to availability of excess nutrients
- As time pass, they increase in number with rapid use of the nutrients and simultaneously produce toxic metabolites
- Due to production of toxic metabolites the growth of organisms slow down during the later stages of the fermentation process

Continuous culture

For achieving the steady state growth, the constant population should be maintained in the medium. This can be achieved by adding new medium and removing few cells at frequent time interval. The population can be maintained in the exponential phase and at a constant biomass concentration for extended periods. This type of culture is referred as **continuous culture.** These can again be categorized into two types: 1. Chemostat and 2. turbidostat

1. Chemostat : Maintaining the population of bacteria at particular stage using an important nutrient source as growth control is referred as chemostat. Ex. In a medium, if the glucose concentration is maintained by subsequent addition at particular dilution rate, so that it will be taken up by the organism immediately and at the same time a portion of the culture will be removed subsequently. This condition will maintain the bacterial growth at particular stage especially in log phase. This kind of devise is called chemostat.
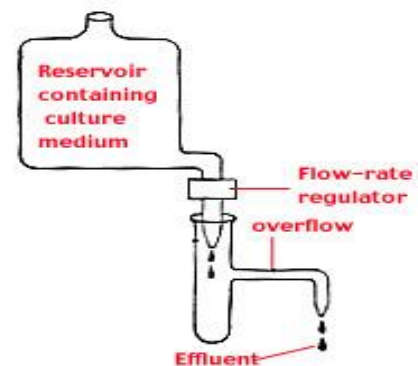
chemostat - model

2. Turbidostat : An electrical devise will monitor the cell density (turbidity) and at appropriate turbidity the nutrient will be added at a dilution rate to maintain the constant population. If the cell density is higher, the instrument will add more quantity of solution to reduce or dilute the population and vice versa. This kind of device is referred as turbidostat.
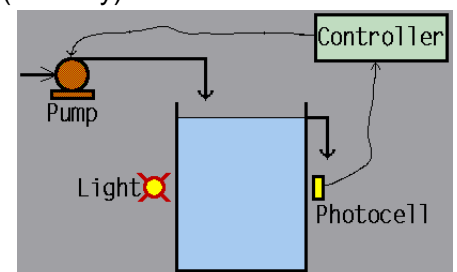

Turbidostat

Measurement of Microbial Growth:

A number of techniques are available in order to measure growth of microbial populations. Either population number of mass may be calculated ad growth leads to increase in both.

1.Direct measurement of cell numbers: Bacteria or microorganisms can be counted directly on the plate and also called as plate counting. Advantage of this method is that it measures the number of viable cells. Disadvantage is that, it is time consuming and expensive as one needs media and other conditions need to be maintained. Bacteria counted on plate counts are referred to as colony forming units as a single cell or a clump of bacterial cells can lead to a colony which contains many cells. The colonies when they are counted in plate count method are to be present sparsely for accurate counting as overcrowding can lead to incorrect counting. To solve this, one has to adapt the serial dilution method in order to get an accurate count.
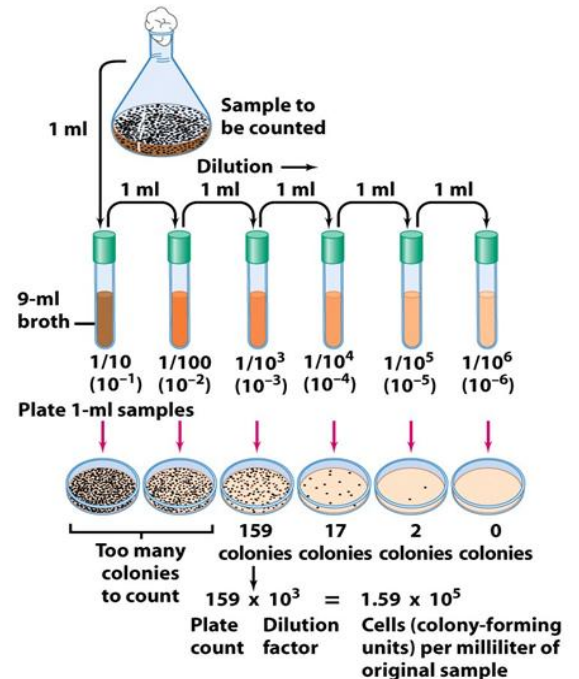
Serial dilution and pour and spread plate: Supposing one has to accurately count the number of cells given in a solution, then serial dilution needs to be performed. A 1ml of the sample is taken and transferred to a tube containing 9ml of sterile water and this process can be repeated until we reach a considerable dilution (say 10^6 to 10^7). Once the original inoculum is diluted one needs to perform a pour plate or a spread plate technique in order to count the number of bacteria present in the diluted sample and then the original sample. In pour plate method the diluted sample is poured into the petriplate and then the medium which is at nearly 50^0C is poured over the inoculum and mixed by gentle agitation. With this method, colonies grow within the nutrient agar as well as on the surface of the agar plate. As certain disadvantages are encountered in this method like heat sensitive microorganisms might not grow and also bacteria when they grow within the nutrient medium might not be useful for diagnostic purposes. In order to avoid these problems, spread plate method is mostly used (Fig. 3). A 0.1ml of the diluted sample is added to the surface of the nutrient medium and spread uniformly with the help of a glass spreader and after incubation, the colonies can be counted and the concentration of the bacterial cells in the original sample is calculated as follows:

Number of bacteria/ml = Number of colonies on plate x reciprocal of dilution of sample

Membrane Filtration: This method can be used in order to study if the quantity of the bacteria is very small as in aquatic samples like lakes, streams etc. Membranes with different pore sizes are used to trap different microorganisms. The sample is drawn through these special membrane filters and placed on an agar medium or on a pad soaked with liquid media. After incubation, the number of colonies can be counted and the number determined in the original sample. Selective media or differential media can be used for specific microorganisms. This is mostly used for analyzing aquatic samples.

Microscopic count: The Petroff-Hausser counting chamber or slide is easy, inexpensive and relatively quick method and also gives information about the size and morphology of the microorganisms. These specially designed slides have chamber of known depth with an etched grid on the chamber bottom (Fig.4).

Bacteria/mm^3 = (bacteria/square) (25 squares)/ (50)

Bacteria can be counted by taking into account the chamber's volume and any sample dilution. The disadvantage encountered in this method is that fairly large volume is required and also it is difficult to distinguish between living and dead cells. Microorganisms of larger sizes can be counted by using electronic counters such as coulter counter; where in the number of cells in a measured volume of liquid is counted. This method gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells.
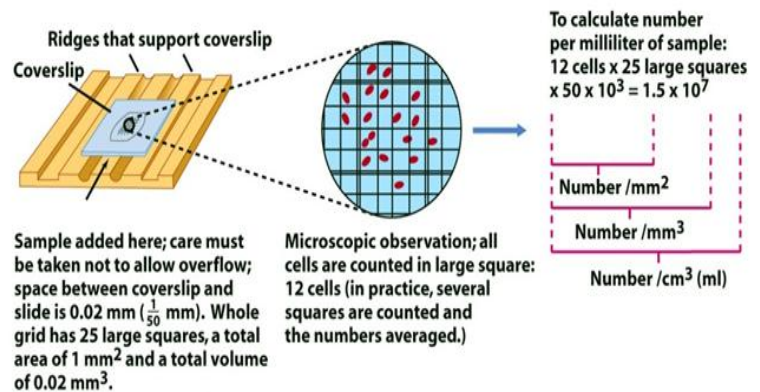

Fig. 4. Direct microscopic count of bacterial cells

2.Indirect methods of measurement of cell mass:
Population growth leads to increase in the total cell mass, as well as in cell numbers. The following methods can be used.

Turbidity: As bacteria grow/multiply in a liquid medium, the medium becomes turbid (Fig. 5). Spectrophotometer is used in order to measure the turbidity. A beam of light is transmitted through a bacterial suspension to a light-sensitive detector. The fact that microbial cells scatter light striking them, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. The extent of light scattering can be measured and is almost linearly related to bacterial concentration at low absorbance levels.
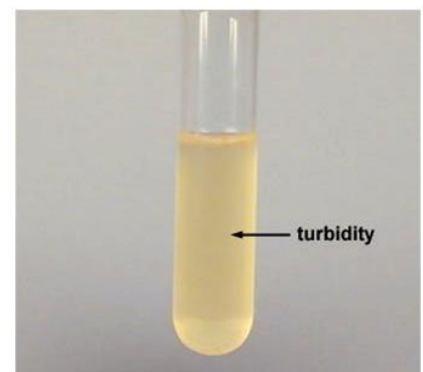

Fig. 5. Broth culture showing turbidity

Dry weight: This method is mostly used for filamentous bacteria and moulds. The microorganism is grown in liquid medium, filtered or centrifuged to remove extraneous material, and dried in an oven and then weighted. It is time consuming and hence not very sensitive.