Growth of bacterial cultures:

Growth is defined as an increase in cellular constituents which leads to a rise in cell number. As we are aware, microorganisms reproduce by binary fission or by budding. In order to study growth, normally one follows the changes in the total population number. The cells copy their DNA almost continuously and divide again and again by the process called **binary fission** . **Binary fission** which has been described earlier a s the form of asexual reproduction in single-celled organisms by which one cell divides into two cells of the same size. Fortunately, few prokaryotic populations can sustain exponential growth for long. Environments are usually limiting in resources such as food and space. Prokaryotes also produce metabolic waste products that may eventually pollute the colony's environment. Still, you can understand why certain bacteria can make you sick so soon after infection or why food can spoil so rapidly. Refrigeration retards food spoilage not because the cold kills the bacteria on food but because most microorganisms reproduce very slowly at such low temperatures.

Binary fission:

In the process of binary fission, the cell elongates and the DNA is replicated. Cell wall and plasma membrane begin to grow inward and cross-wall forms completely around the divided DNA. At the end the cell separates into two individual cells similar to the parent cell and contains all the contents a cell requires for its living including DNA (Figure 1).

Few bacterial species reproduce by budding. In this method, the cell forms a small initial growth, it enlarges and then it separates from the parent cell. Some filamentous bacteria like actinomycetes reproduce by producing chains of conidiospores and a few bacteria simply fragment.

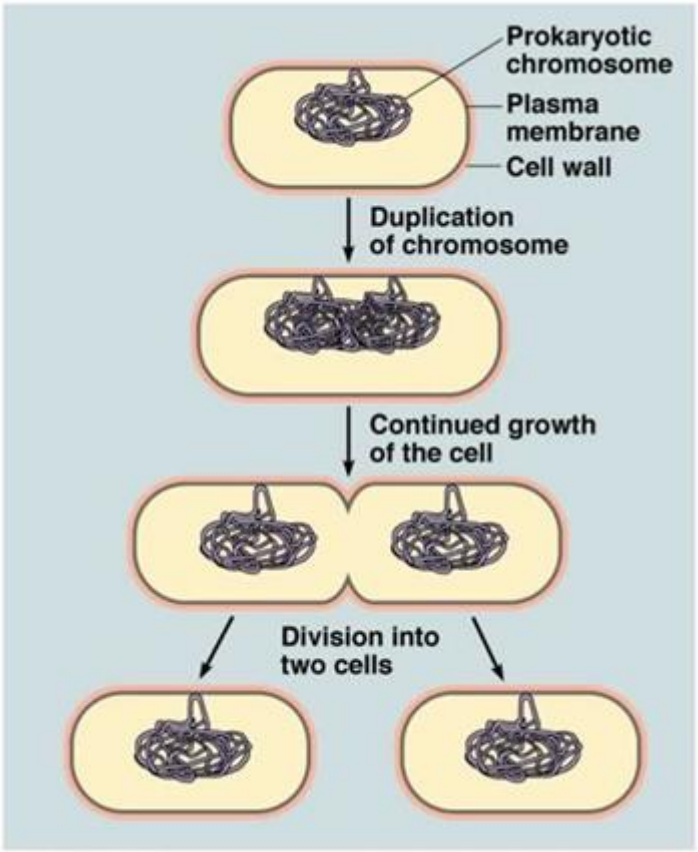

Fig. 1. Binary fission in bacteria

Growth Curve:

The increase in cell number or growth in population is studied by analyzing the growth curve of a microbial culture. Bacteria can be grown or cultivated in a liquid medium in a closed system or also called as batch culture. In this method, no fresh medium is added and hence with time, nutrient concentration decreases and an increase in wastes is seen. As bacteria reproduce by binary fission, the growth can be plotted as the logarithm of the number of viable cells verses the time of incubation. The curve plotted shows four basic phases of growth; the lag, log, stationary, and death phase (Figure 2).

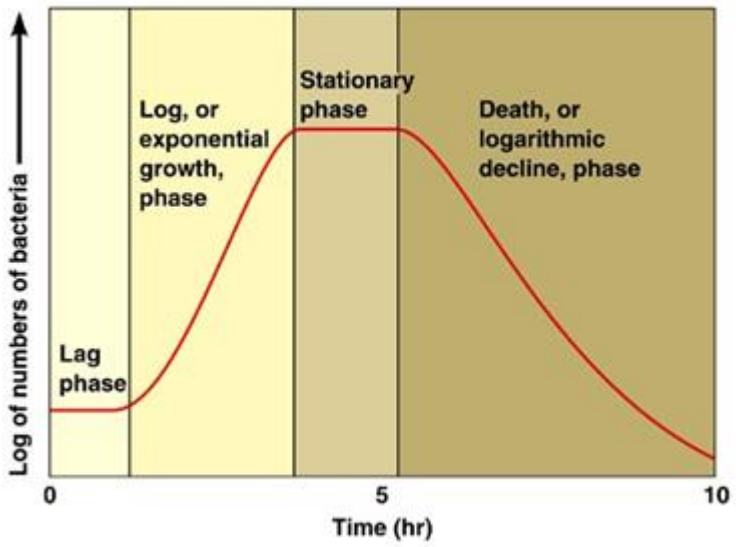

Fig. 2. Growth curve of a typical bacterial cell

Lag Phase: As the cells are introduced into the new medium, no immediate increase in cell number occurs. During this phase, the cells are undergoing a period of intense metabolic activity involving synthesis of enzymes and various other molecules required to divide in the coming phase. This phase can vary considerably in length depending on the nature of the medium and the microorganism. The medium may be different from the one the microorganism was growing in previously. The cells may be old and depleted of ATP, essential cofactors and ribosomes; these must be synthesized before growth can begin. So, the microorganism requires time to recover and young, vigorously growing cultures and fresh medium are to be used for the lag phase to be short.

Log Phase: In this phase the cell starts dividing in a logarithmic way and this is also called as exponential phase and the growth is balanced. Cellular reproduction is high during this period and the plot during this phase is a straight line. The cells are most active metabolically during this phase and the population is most uniform during this phase; therefore exponential phase cultures are usually used in biochemical and physiological studies. But during this phase, the microorganisms may be particularly sensitive to adverse conditions. On the whole, in this phase the cells are growing and dividing and increasing in cell number.The rate of exponential growth of a bacterial culture is expressed as **generation time** , also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation/ n (n = number of generations). Hence, G=t/n is the equation from which calculations of generation time can be derived.

Exponential phase or log phase is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, unbalanced growth results. The individual cells may take slightly longer than

others to go from lag phase to the log phase, and they do not all divided precisely together. If they divided together and the generation time is same, the number of cells in a culture would increase in a stair – step pattern, exactly doubling every 20 min or a particular time – a hypothetical situation called Synchronous growth. In an actual culture, each cell divides sometime during the 20 min generation time, with about 1/20 cells dividing each minute – a natural situation called nonsynchronous growth or asynchronous growth which appears as a smooth line, not as steps.

Organisms in a tube of culture medium can maintain logarithmic growth for only a limited time. As the number of organisms' increases, nutrients are used up, metabolic wastes accumulate, living space may become limiting factor and aerobes suffer from oxygen depletion

Stationary Phase: Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask). Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space".During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process. Starving bacteria frequently produce a variety of starvation proteins, which make the cell much more resistant to damage. They increase peptidoglycan cross-linking and cell wall strength. The Dps (DNA- binding proteins from starved cells) protein protects the DNA. Bacterial pathogens like *Salmonella typhymurium* become more virulent when starved.

Death Phase: Due to the conditions during the stationary phase, the death phase is seen as there is a decline in the number of viable cells. This phase also is like the log phase where the cell number is declining in a logarithmic way. The cell is said to be dead if it does not revive itself and reproduce when incubated again in a fresh medium. In this phase, the number of live cells decreases at a logarithmic rate, as indicated by the straight downward sloping diagonal line. The duration of this phase is as highly variable as the duration of log phase. Both depend primarily on the gen **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)

$b = B \times 2^n$ (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

etic characteristics of the organism.

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.

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

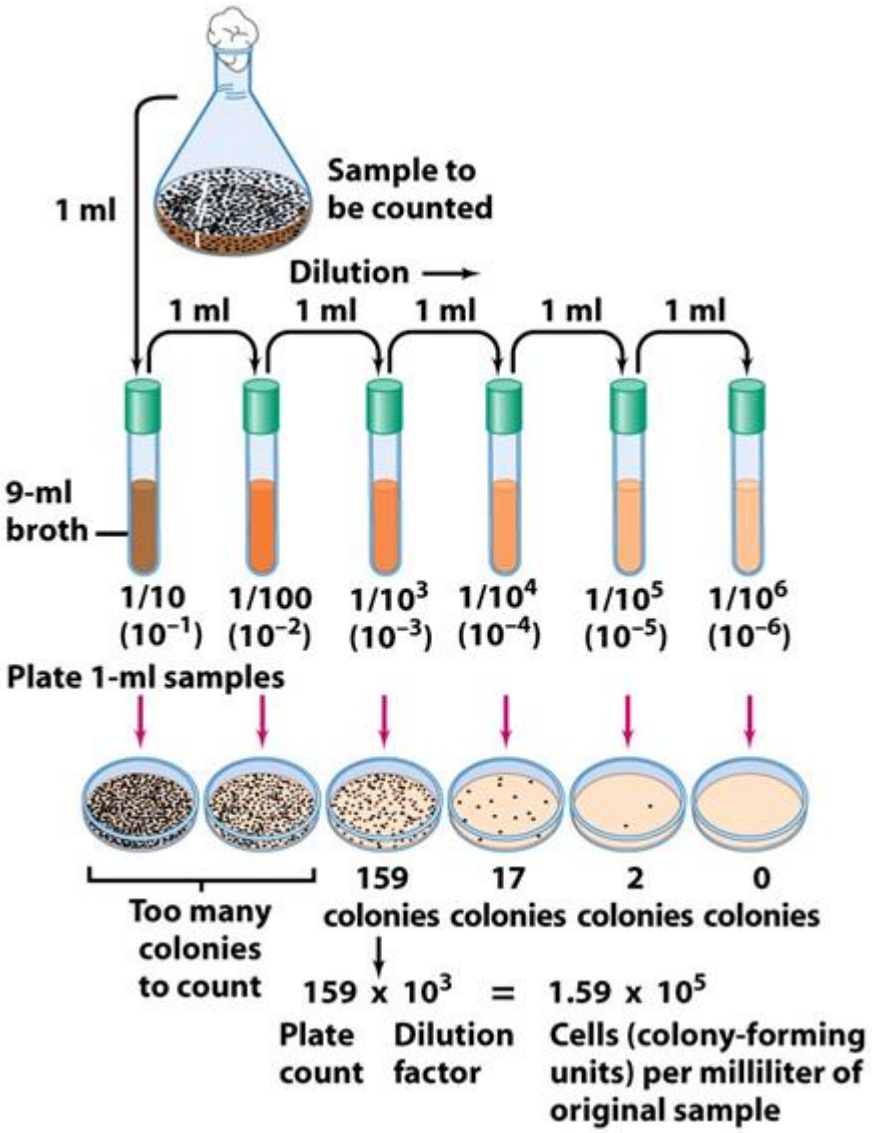

Fig. 3. Serial dilution methodology

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.

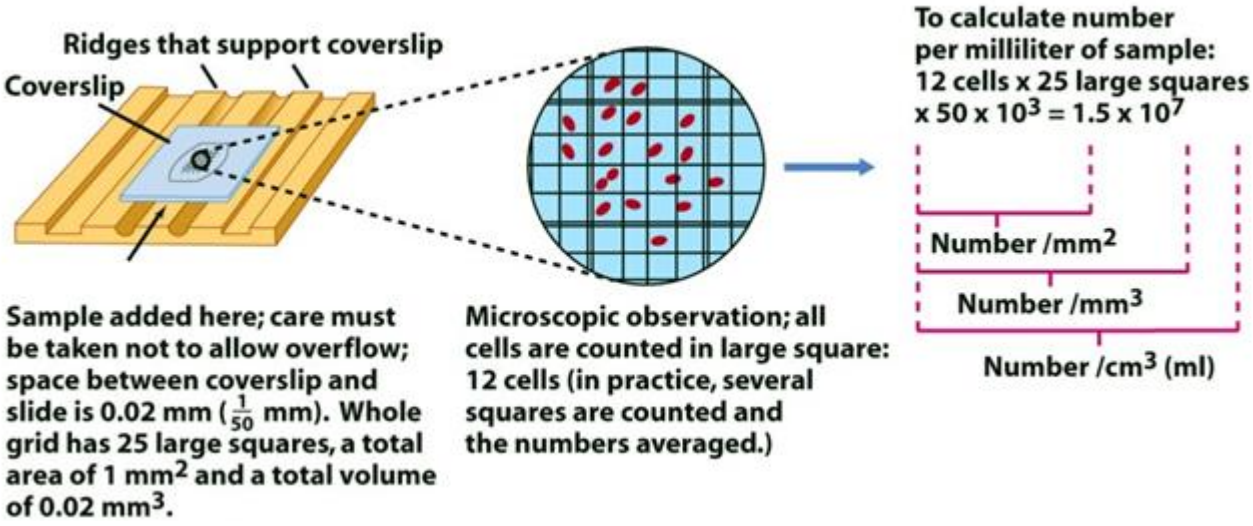

Fig. 4. Direct microscopic count of bacterial cells

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.

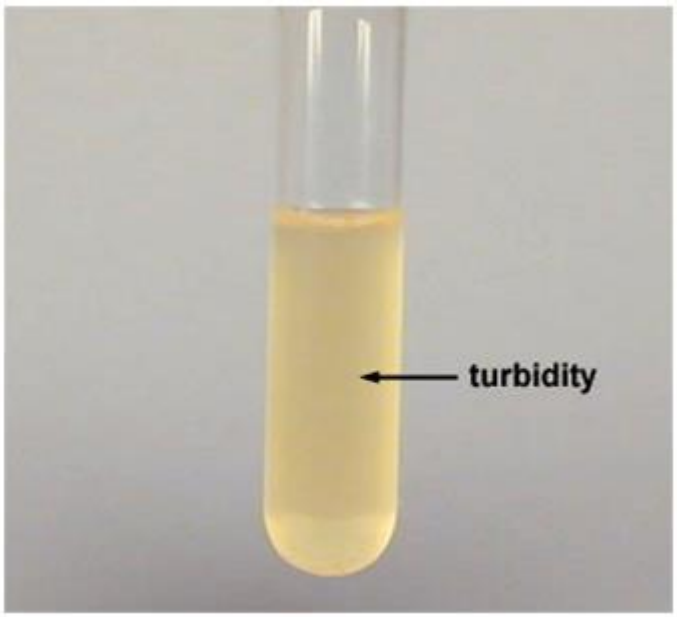

Fig. 5. Broth culture showing turbidity

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.

Continuous culture of Microorganisms:

Batch cultures : Nutrient supplies are not renewed nor wastes removed.

Continuous cultures : continuous provision of nutrients and removal of wastes takes place. The population can be maintained in the exponential phase and at a constant biomass concentration for extended periods.

These can again be categorized into two types:

Chemostat : Where sterile medium is fed into the culture vessel at the same rate as the media containing microorganisms is removed.

Turbidostat : It has a photocell that measures the absorbance or turbidity of the cell culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density.

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. An understanding of these influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms. Prokaryotes are present or grow anywhere life can exist. The environments in which some prokaryotes grow would kill most other organisms. For example *Bacillus infernus* is able to live over 1.5 miles below the earth's surface without O_2 and 60°C temperature. These microorganisms which can thrive and grow in such harsh conditions are often called **extremophiles.**

The major physical factors which affect microbial growth are solutes and water activity, pH, temperature, oxygen level, pressure and radiation.

Solutes and Water activity: Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings. Microorganisms need to keep the osmotic concentration of their cytoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. In a hypertonic environment, the prokaryotes increase their internal osmotic concentration through the synthesis or uptake of choline, proline, glutamic acid and other amino acids. A few prokaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions. The enzymes of these bacteria are altered for the requirement of high salt concentrations for normal activity. Halophiles grow optimally in the presence of NaCl or other salts at a concentration above about 0.2M. These have extensively modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes. They require higher potassium levels for stability and activity. The plasma membrane of halophiles is also stabilized by high concentration of sodium ions.

Water activity (a_w) is the amount of water available to microorganisms and this can be reduced by interaction with solute molecules (osmotic effect). Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's a w is low. Microorganisms differ greatly in their ability to adapt to habitats with low water activity. In a low a w habitat, the microorganisms

must expend extra effort to grow as it should maintain a high solute concentration to retain water. Such microorganisms are osmotolerant or can grow over wide range of water activity or osmotic concentration. Most of the microorganisms grow at a w =0.98 or higher.

pH: It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$pH = -\log [H^+] = \log (1/H^+)$

The pH scale ranges from 1.0 to 14.0 and most microorganisms grow vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH values between 9.0 and 10. The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity. *Cyanidium caldarium* (algae) and archaeon *Sulfolobus acidocaldarium* are inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperature. Drastic changes/variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Prokaryotes die if the internal pH drops much below 5.0 to 5.5. External pH alterations also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. The microorganism needs to maintain a neutral cytoplasmic pH and for this the plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkalophiles maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. The antiport systems probably correct small variations in pH. In case of too much acidity (below 5.5 to 6.0) *S. typhimurium* and *E.coli* synthesize an array of new proteins as part of what has been called as their acidic tolerance response. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. Microorganisms can change the pH of their own habitat by producing acidic or basic metabolic waste products. In order to maintain the pH, buffers are often included in the media to prevent growth inhibition. Phosphate is commonly used buffer and a good example of buffering agent. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature: Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. Beyond a certain point of higher temperature, slow growth takes place and damages the microorganisms by denaturing enzymes, transport carriers and other proteins. The plasma membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don't work rapidly. In summary, when organisms are above their optimum temperature, both the function and cell structure is affected at low temperature, function is affected. The cardinal temperatures vary greatly between microorganisms. Optimum usually range from 0°C to as high as 75°C, where as microbial growth occurs at temperature extending from -20°C to over 120°C. Archaen *Geogemma barossii* grows anaerobically at 121°C. The major microbial groups differ from one another regarding their maximum growth temperature. Upper limit for protozoans is around 50°C, some algae and fungi can grow at temperatures as high as 55°C to 60°C.

Did you know: Prokaryotes have been found growing at or close of 100°C. Now thermophilic prokaryotes have been reported growing in surface of chimneys or black smokers located along rifts and ridges on the ocean floor that spew sulphide-rich super-heated vent water with temperatures above 350°C. These microbes can grow and reproduce at or above 112°C. The proteins, membranes and nucleic acids of these prokaryotes are remarkably temperature stable and provide ideal subjects for studying the ways in which macromolecules and membranes are

stabilized. Some thermostable enzymes from these organisms have important industrial and scientific uses. For ex., the Taq polymerase from the thermophilic *Thermus aquaticus* is used extensively in the polymerase chain reaction.

Microorganisms are classified into five classes based on their temperature ranges for growth.

1. 1. **Psychrophiles:** Microorganisms grow well at 0°C and the optimum growth temperature of 15°C or lower and maximum at around 20°C. These microorganisms are isolated from Arctic and Antarctic habitats. They have adapted to their environment in several ways. Their enzymes, transport systems and protein synthetic mechanisms function well at low temperatures. The cell membranes have high levels of unsaturated fatty acids and remain semifluid when cold. At higher than 20°C, the psychrophiles begin to leak cellular constituents because of cell membrane disruption. Microorganisms such as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium* belong to this group. The psychrophilic *Chlamydomonasnivalis* turns a snowfield or glacier pink with its bright red spores.

   2. **Psychrotrophs or Facultative Psychrophiles:** In this group many **s** pecies can grow at0 o C to 7°C, optimum between 20°C and 30°C. The spoilage of refrigerated foods is mainly caused by microorganisms belonging to this group.

   3. **Mesophiles:** Growth optimum around 20°C to 40°C, minimum at 15°C to 20°C and maximum at 45°C or lower. Most of the organisms fall under or within this category including human pathogens.

   4. **Thermophiles:** The microorganisms in this group can grow at temperature of 55°C or higher, minimum is usually around 45°C and growth optima at around 55°C to 65°C. Mostly prokaryotes and a few algae and fungi belong to this group. The habitats in which they grow include, composts, self-heating haystacks, hot water lines and hot springs. Microorganisms have more heat-stable enzymes and proteins synthesis systems, which function at high temperature. Heat stable proteins have high organized, hydrophobic interiors, more hydrogen bonds and other non-covalent bonds strengthen the structure. Amino acids like proline make the polypeptide chain less flexible and chaperones also aid in folding of proteins to stabilize them. DNA also is stabilized by specific histone like proteins. The membrane lipids are also stable and tend to be more saturated, more branched and of higher molecular weight. Archaeal thermophiles have membrane lipids with ether linkages, which protect the lipids from hydrolysis at high temperatures.

   5. **Hyperthermophiles:** Few microorganisms can grow at 96°C or above and have maximum at 100°C; and growth optima between 80°C and about 113°C. *Pyrococcus* and *Pyrpdictiumoccultum* are examples of marine hyperthermophiles found in hot floors of the sea floor.

   Oxygen Concentration: An aerobe is an organism able to grow in the presence of atmospheric O_2 and the ones that grow in its absence is an **anaerobe**. Organisms which completely are dependent on atmospheric O_2 for growth are **obligate aerobes**, and it serves as the terminal electron acceptor for the electron transport chain in aerobic respiration and employs it in the synthesis of sterols and unsaturated fatty acids. Organisms which do not require O_2 for growth but do grow better in its presence are called **facultative anaerobes** . **Aerotelerant anaerobes** such as *Enterococcus faecalis* simply ignore O_2 and grow equally well whether it is present or not. **Obligate anaerobes** like *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus, Neocallimastix*, do not tolerate O_2 at all and die in its presence. Aerotelerant and obligate anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for the purpose. **Microaerophiles** are those organisms that are damaged by the normal atmospheric levels of O_2 (20%) and require O_2 levels between the range of 2% to 16% for growth. The nature of bacterial O_2 responses can be readily determined by

growing the bacteria in culture tubes filled with a solid culture medium or a special medium like thioglycollate broth, which contains a reducing agent to lower O_2 levels (Fig. 6). Aerobic microorganisms are cultured, either the culture vessel is shaken to aerate the medium or sterile air is pumped. Anaerobic microorganisms require special anaerobic media containing reducing agents such as thioglycollate or cysteine may be used. Removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas is also preferred. Co_2 and nitrogen is added to the chamber since many anaerobes require a small amount of Co_2 for best growth. The technique in which gas pak jar is used can be used.

Prokaryotes and protozoa are found arranged among all the 5 types of microorganisms. Fungi are normally aerobic, but species particularly among yeasts, are facultative anaerobes. Algae are almost always obligate aerobes. The different relationships with O_2 appear due to several factors, including the inactivation of proteins and the effect of toxic O_2 derivatives. Enzymes can be inactivated when sensitive groups like sulfydryls are oxidised. A notable example is the nitrogen fixation enzymes nitrogenise which is very O_2 sensitive.

Oxygen accepts electrons and is readily reduced because its two outer orbital electrons are unpaired. The reduction products such as superoxide radical, hydrogen peroxide and hydroxyl radical can be resulted by flavoproteins, several other cell constituents and radiation.

$$O_2 + e^- \rightarrow O_2^{-\bullet}$$

$$O_2^{-\bullet} + e^- + 2H^+ \rightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH^\bullet$$

These are extremely toxic because they are powerful oxidizing agents and rapidly destroy cellular constituents. As microorganisms can be killed, they need to protect themselves from such oxygen products. Microorganisms possess enzymes that afford protection against toxic O_2 products. Obligate aerobes and facultative anaerobes usually contain the enzymes superoxide dismutase (SOD) and catalase, which catalyze the destruction of superoxide radical and hydrogen peroxide respectively. Peroxidase also can be used to destroy hydrogen peroxide.

$$2O_2^{-\bullet} + 2H^+ \rightarrow O_2 + H_2O_2 \text{ (Superoxide dismutase)}$$

$$2H_2O_2 \rightarrow 2H_20 + O_2 \text{ (Catalase)}$$

$$H_2O_2 + NADH + H+ \rightarrow 2H_2O + NAD^+ \text{ (Peroxidase)}$$

Aerotolerant microorganisms may lack catalase but almost always have superoxide dismutase. Strict anaerobes lack either of the enzymes or have them in very low concentrations and therefore cannot tolerate O_2. Aerobic microorganisms can be grown in an aerated medium which is aerated by shaking the vessel or sterile air is pumped. But for anaerobes, O_2 must be excluded from the medium. Reducing agents such as thioglycollate or cysteine can be used to flush out O_2. Nitrogen gas can also be used to eliminate O_2 with a vacuum pump, and sometimes CO_2 and nitrogen is added to the chamber, as anaerobes require a small amount of CO_2 for best growth and also gas pack jar can also be used.

Pressure: Most microorganisms always are subjected to pressure of 10 atmospheres (atm). The hydrostatic pressure can reach to 600 to 1100 atm in the deep sea with temperature about 2°C to 3°C. Organisms can survive and adapt at these extreme conditions and many are barotolerant,

increased pressure does adversely affect them but not as much as it does to nontolerant bacteria. The barophillic organisms are those growing in the guts of deep sea invertebrates such as amphipods and holothurians and grow more rapidly at high pressures. These bacteria may play an important role in nutrient recycling in the deep sea. Bacterial genera of *Photobacteria, Shewanella, Colwellia* are barophiles. Some members of the Archaea are thermophiles for example *Pyrococcus spp., Methanococcus janaschii* .

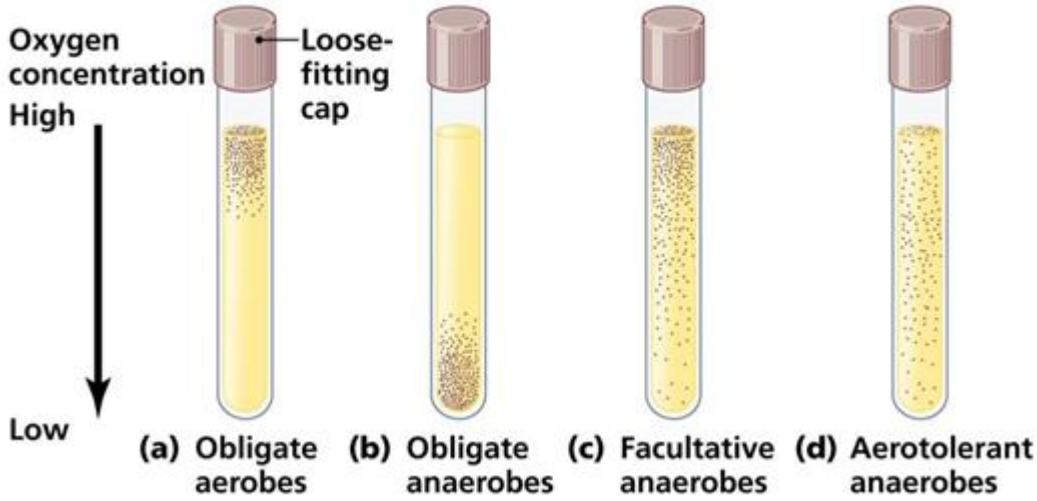

Fig. 6. Oxygen requirements in bacteria

Pressure:

Most organisms on land or on the surface of water is always subjected to a pressure of 1 atm. The hydrostatic pressure can reach 600 to 1100 atm in the deep sea. Despite these extremes, bacteria survive and adapt. Many are barotolerant. Some bacteria in the gut of deep sea invertebrates such as amphipods and holothurians are truly barophilic and grow more rapidly at high pressures (Ex. *Photobacterium, shewanella, Colwellia*).