Factors affecting microbial Growth

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^oC 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.

Some 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, E.g. 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. **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* .

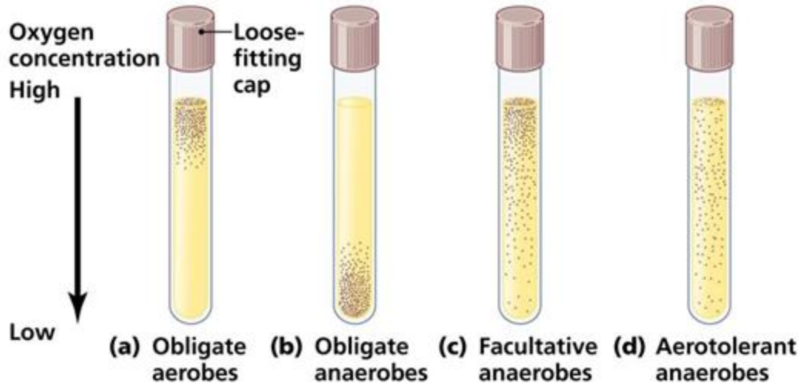

Fig. Oxygen requirements in bacteria

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*).

Radiation:

Electromagnetic radiation of various types bombards our world. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases – gamma rays and X rays are much more energetic than visible light or infrared waves (Fig. 7). Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation, infrared rays and radio waves. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun as visible light. Many forms of electromagnetic radiation are very harmful to microorganisms. Ionizing radiation, radiation of very short wavelength or high energy can cause atoms to lose electrons or ionize. The two major forms of ionizing radiation, X rays which are artificially produced and gamma rays which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations, higher levels are directly lethal. Some prokaryotes like *Deinococcus radiodurans* and bacterial endospores are resistant and can cause a variety of changes in cells like; it breaks hydrogen bonds, oxidises double bonds, destroys ring structures and polymerizes some molecules. Oxygen enhances these destructive e effects, probably through the generation of hydroxyl radicals (OH.). Destruction of DNA is the most important cause of death of microorganisms. Ultraviolet radiation kills all kinds of microorganisms due to its short wavelength (approximately 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. Formation of thymine dimmers in DNA is the primary mechanism of UV damage; these dimmers inhibit DNA replication and function. This damage is repaired by photo reactivation, where blue light is used by a photo reactivating enzyme (photolyase) to split the thymine dimmers. Dark reactivation, where a short sequence containing the thymine dimmers can also be excised and replaced in the absence of light. Damage can also be repaired by the recA protein in recombination repair and SOS repair.

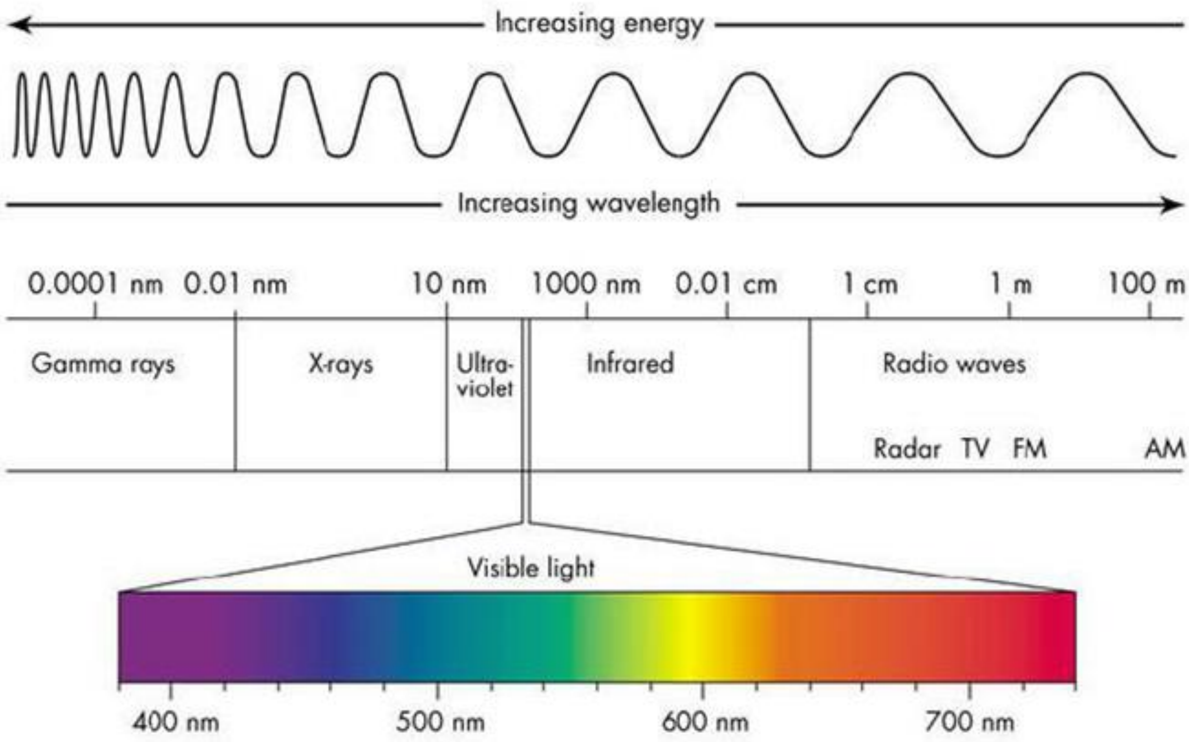

Fig Electromagnetic spectrum

Visible light – immensely beneficial becuas eit is the source of energy for photosynthesis. Visible light when present in sufficieint intensity can damage or kill microbial cells. Pigments called photosensitizers and O_2 are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophull, cytochromes and flavins which absorb light energy, become exited or activated and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O_2 generating singlet oxygen (‘O_2).

$$P\ (light) \rightarrow P(activated)$$

$$P(activated) + O_2 \rightarrow P + {}^{\prime}O_2$$

Singlet oxygen is very reactive, powerful oxidizing agent that will quickly destroy a cell. Many microorganisms that are airborne or live on exposed surface use carotenoid pigments for protection against photooxidation. Carotenoids effectively quench singlet oxygen that is absorbing energy from singlet oxygen and convert it back into the unexcited ground state.

Reference: Open access source