Microorganisms are similar to more complex organisms in that they need a variety of materials from their environment to function and accomplish two primary goals--supply enough energy to manage their processes and extract building blocks to repair themselves or procreate. In addition to what they take in, microorganisms also thrive in particular environments. These environments vary as much as the organisms do themselves, and even the amount and distribution of elements in any particular environment can be very important.

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 Acidity:

Water is one of the most essential requirements for life. Thus, its availability becomes most important factor for the growth of microorganisms. The availability of water depends on two factors — the water content of the surrounding environment and the concentration of solutes (salts, sugars, etc.) dissolved in the water.

In most cases, the cell cytoplasm possesses higher solute concentration in comparison to its environment. Thus, water always diffuses from a region of its higher concentration to a region of the lower concentration.

This process is called osmosis which keeps the microbial cytoplasm in positive water balance. When a microbial cell is placed in hypertonic solution (or, solution of low water activity) it loses water and shrinkage of membrane takes place. This phenomenon is called plasmolysis.

Microorganisms show variability in their ability to adapt the habitats of low water activity. Microorganisms like S. aureus can survive over a wide range of water activity and are called as osmotolerant (as water activity is inversely related to osmotic pressure).

However, most microorganisms grow well only near pure water activity (i.e., around 0.98-1). Thus, drying of food or addition of high concentration of salts and sugars is the most popular way of preventing spoilage of food.

Seawater microorganisms are called as halophiles since they require high concentration of salts (between 2.8-6.2 M) to grow. Halobacterium, a halophilic archaebacterium, inhabits Dead Sea (a salt lake situated between Israel and Jordan and the lowest lake in the world), the Great Salt Lake in Utah and other aquatic habitats possessing salt concentrations approaching salt water.

The quantitative availability of water can be expressed in physical term called water activity (a_w). The water activity for a sample solution is the ratio of vapour pressure of the sample solution to the vapour pressure of the water at the same temperature ($a_w = P_{soln}/P_{water}$).

The relative humidity of a test sample (at equilibrium) can be obtained, after sealing it in a closed chamber. This determines the water activity of a solution. For example, if after treating by above method relative humidity of air over the sample is 95% then, the water activity of the sample is 0.95.

Temperature:

All forms of life are greatly influenced by temperature. In fact, the microorganisms are very sensitive to the temperature since their temperature varies with that of environment (puikilothermic).

Temperature influences the rate of chemical reactions and protein structure integrity thus affecting rates of enzymatic activity. At low temperature enzymes are not denatured, therefore, every 10°C rise in temperature results in rise of metabolic activity and growth of microorganisms.

However, enzymes have a range of thermal stability and beyond it their denaturation takes place. Thus, high temperature kills micro-organism by denaturing enzymes, by inhibiting transport carrier molecules or by change in membrane integrity. Each microbe shows characteristic temperature dependence and possesses its own cardinal temperatures i.e. minimal, maximum and optimal growth temperatures (Fig. 19.15).

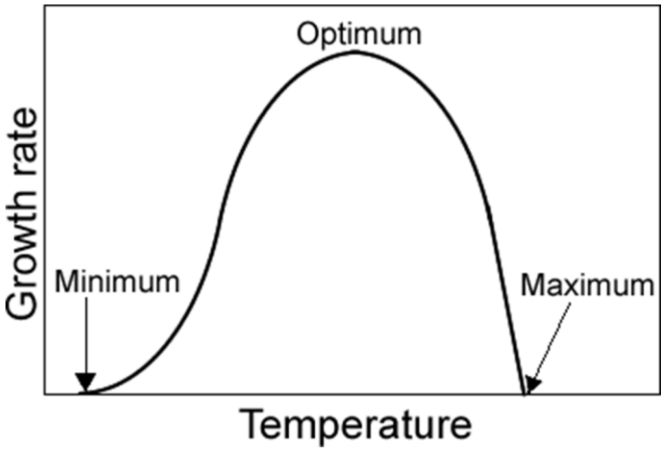

Fig: Cardinal temperature

The values for cardinal temperature vary widely among bacteria. For convenience, bacteria isolated from hot springs can survive even at temperature of 100°C and above, while those isolated from snow can survive below -10°C. On the basis of susceptibility to the thermal conditions, microorganisms are classified into three categories: thermophiles, meshophiles and psychrophiles.

Thermophiles are microorganisms that show growth optima at 55°C. They often have growth maxima of 65°C, while few can grow even at 100°C and higher temperatures. Their growth minima are 45°C. The vast majority of thermophiles belong to prokaryotes although a few microalgae (e.g., Cyanidium caldarium) and micro fungi (e.g. Mucor pusillus) are also thermophiles.

A few microorganisms are hyperthermophiles as they possess growth optima between 80°C and about 113°C. Hyper-thermophiles usually do not grow well below 55°C (e.g., Pyrococcus abyssi, Pyrodictium occultum).

Mesophiles are microorganisms that have growth minima between 15°C-20°C; optima between 20-45°C and maxima at 45°C. Most microorganisms fall within this category. Almost all human pathogens are mesophiles as they grow at a fairly constant temp, of 37°C.

Psychrophiles have optimum temperature for growth at 15°C; however, few can grow even below 0°C.

The maximum growth temperature of psychrophiles is around 20°C. The spoilage of refrigerated food takes place because of facultative psychrophiles. These are microorganisms that can grow at 0°C but have growth optima temperature between 20°-30°C.

pH:

The pH is defined as a negative logarithm of hydrogen ion concentration:

The activity of microbial enzymes depends on the change present on the surface of amino acids. Any change in the environmental pH may either enhance the enzyme activity or inhibit the activity (Table 19.2).

TABLE 19.2. pH range of few bacteria

Organisms	Minimum	Optimum	Maximum
Thiobacillus thiooxidans	1.0	2.0–2.8	4.0–6.0
Lactobacillus acidophilus	4.5–4.6	5.8–6.6	6.8
Clostridium sporogens	5.0–5.8	6.0–7.6	8.5–9.0
Pseudomonas aeroginosa	5.6	6.6–7.0	8.0
Nitrobacter sp.	6.6	7.6–8.6	10.0
Nitrosomonas sp.	7.0–7.6	8.0–8.8	9.4

Thus, pH can dramatically affect the growth of microorganisms. Each species of microorganisms shows specific pH growth range. Microorganisms can be classified as acidophilus, neutrophils and basophiles (alkalophiles) on the basis of their requirement for particular pH in their environment.

The acidophiles grow between the pH range of 0.0 to 5.5, neutrophils between 5.5 to 8 and basophiles between 4.5 to 11.5. Most micro fungi are acidophiles as they grow in surroundings having pH about 4 to 6. Most bacteria and protozoa are neutrophils.

Growing microorganisms produce acidic and basic metabolic waste products. These wastes often become inhibitory agents as they alter the pH of surrounding environment.

Thus, phosphate or citrate buffers are added to maintain constant pH of the medium. Buffers are salts of weak acids or bases that keep the pH of the medium constant. Phosphate is a commonly used buffer and represents a good example of buffering by a weak acid ($H_2PO_4^-$) and its conjugate base (HPO_4^{2-}) in the following manner.

$H^+ + H^+PO_4^{-2} \rightarrow H_2PO_4^-$

$OH^- + H_2PO_4 \rightarrow HPO_4^{-2} + HOH$

<u>Oxygen Requirements:</u>

The atmosphere of earth contains about 20% (v/v) of oxygen. Microorganisms capable of growing in the presence of atmospheric oxygen are called aerobes whereas those that grow in the absence of atmospheric oxygen are called as anaerobes.

The micro-organisms that are completely dependent on atmospheric oxygen for growth are called obligate aerobes whereas those that do not require oxygen for growth but grow well in its presence are called as facultative anaerobes.

Aerotolerants (e.g. Enterococcus faecalis) ignore O_2 and can grow in its presence or absence. In contrast, obligate anaerobes (e.g., Bacteroids, Clostridium pasteurianum, Furobacterium) do not tolerate the presence of oxygen at all and ultimately die. Few microorganisms (e.g., Campylobacter) require oxygen at very low level (2-10%) of concentration and are called as microaerophiles (Fig. 19.16). The latter are damaged by the normal atmospheric level of oxygen (20%).

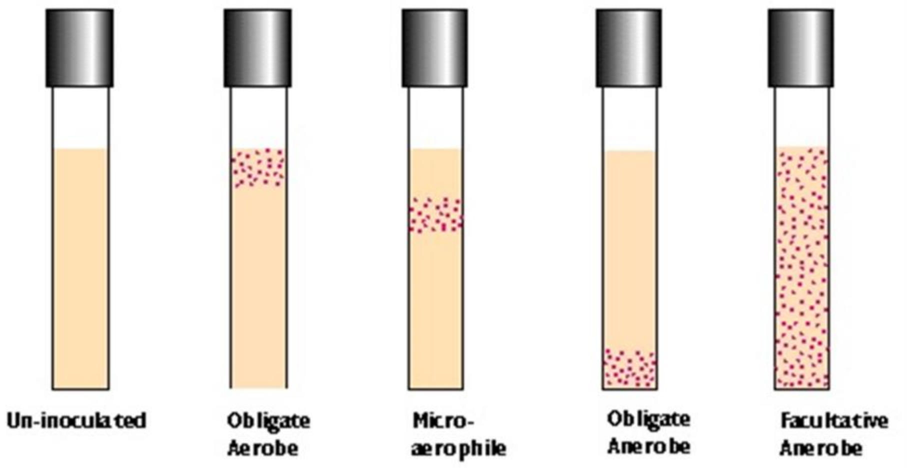

Fig: Relation of oxygen and growth (shake tubes method)

These varying relationships between microbes (especially the bacteria) and O_2 appear due to different factors such as protein-inactivation and the effect of toxic oxygen-derivatives. Bacterial enzymes can be inactivated when interact with oxygen. Nitrogen-fixing enzyme introgenese is very sensitive to oxygen and represents a good example of interaction between enzyme and oxygen.

During metabolic process, flavoprotein reduces oxygen to form hydrogen peroxide (H_2O_2), superoxide radical (O_2^-) and hydroxy radical (OH^-). These compounds are highly toxic and being powerful oxidizing agent, can cause destruction of cellular macromolecules.

In order to survive, therefore, bacteria must be able to protect it from oxidising agents. All aerobes and facultative anaerobes contain two enzymes namely superoxide dismutase and catalase. These enzymes protect microbes against lethal effects of oxygen products.

The oxidizing property of superoxide is nullified by superoxide dismutase as it converts superoxide into oxygen and hydrogen peroxide. The enzyme catalase decomposes hydrogen peroxide into oxygen and water. The aero-tolerant bacteria like Lactic acid bacteria possess enzyme peroxidases instead of catalase to decompose the accumulated hydrogen peroxide.

$$2O_2^- + 2H^+ \xrightarrow[\text{dismutase}]{\text{superoxide}} O_2 + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$H_2O_2 + NADH + H^+ \xrightarrow{\text{Peroxidase}} 2H_2O + NAD^+$$

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Since all obligate anaerobes lack these enzymes or have them in very low concentration and, therefore, are susceptible to oxygen.

Pressure:

Normal life of microorganisms on land or on the water surface is always subjected to a pressure of 1 atmosphere. But, they are many microbes that survive in extremes of hydrostatic pressure in deep sea. Others are there that not only survive rather grow more rapidly at high pressures (e.g., Protobacterium, Colwellia, Shewanella) and are called barophilic.

Some archaebacteria are thermobarophiles (e.g., Pyrococcus spp., Methanococcus jannaschii). However, A barophile has been recovered from the depth about 10,500 m in sea near Philippines and has been found unable to grow at 2°C temp, and below about 400-500 atmospheric pressure.

Radiation:

Some electromagnetic radiations, particularly the ionizing radiation (e.g., X-rays, gamma rays) are very harmful to microbial growth. Low levels of these radiations may cause mutations and may indirectly result in death whereas high levels may directly cause death of the microbes.

Ionizing radiation, however, destroys ring-structures, breaks hydrogen bonds, oxidizes double bonds and polymerizes certain molecules. Ultraviolet (UV) radiation is lethal to all categories of microbial life due to its short wavelength and high-energy; the most lethal UV radiation has a wavelength of 260 nm.

Ultraviolet radiation primarily forms thymine dimers in DNA to cause damage. Two adjacent thymines in a DNA strand join each other covalently and inhibit DNA replication and function. Microbial photosynthetic pigments (chlorophyll, bacteriochlorophyll, cytochromes and flavins), sometimes, absorb light energy, become excited or activated, and act as photosensitizers.

The excited photosensitizer (P) transfers its energy to oxygen which then results in singlet oxygen ($^1O_2$). The latter is very reactive and powerful oxidizing agent and quickly destroys a cell. The singlet oxygen is probably the main weapon employed by phagocytes to destroy engulfed bacteria.

Elements Present:

In addition to water, microorganisms usually require the presence of certain elements in the air--gases that they absorb to produce needed nutrients. Nitrogen is one necessary element, as is oxygen. There are many microorganisms that require an oxygen-rich environment to survive, but others actually flourish in low-oxygen surroundings. Between these two extremes is a wide variety that may prefer more or less oxygen and that will be able to flourish equally no matter how much oxygen is present.