Dr. Ariyah Terasawat

Microbial Growth

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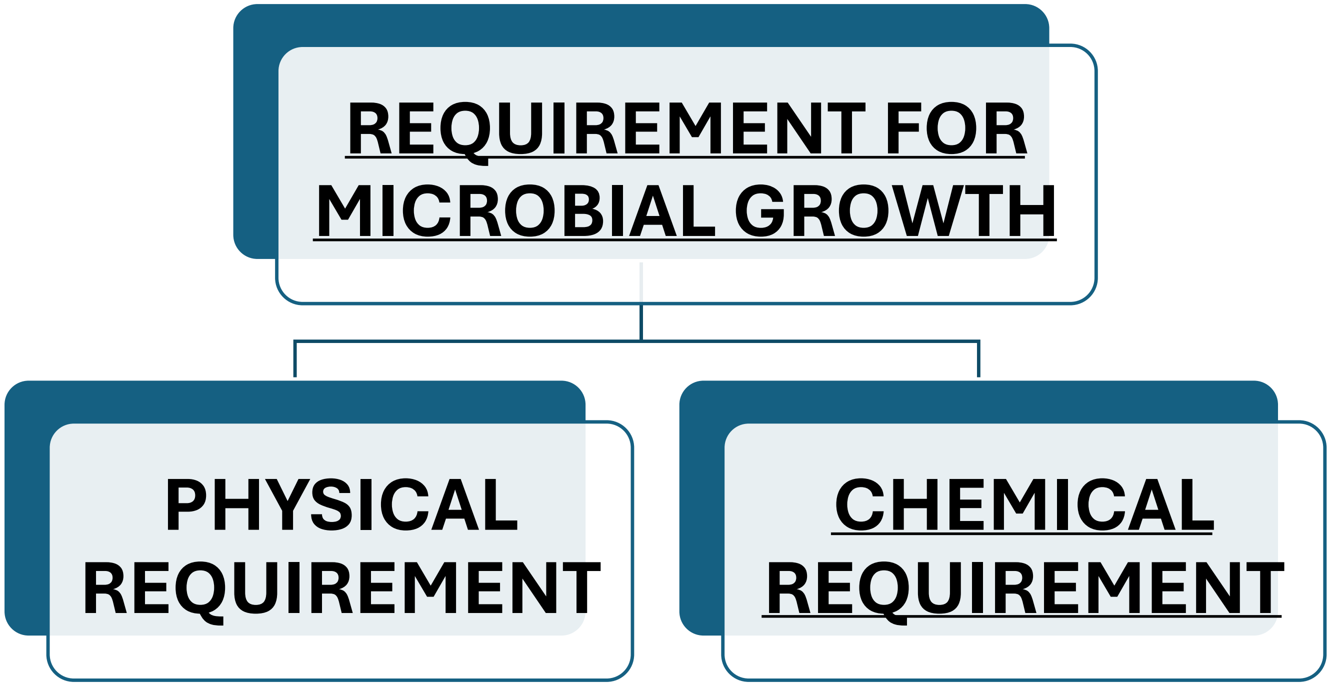
INTRODUCTION

- What is Microbial GROWTH

Living organisms grow and reproduce. The growth indicates that an organism is in active metabolism. In plants & animals, we can see the increase in height or size. Growth common refers to increase in size but with microorganisms particularly bacteria, this term refers to changes in total population rather than increase in size or mass of individual organisms. The change in population in bacteria chiefly involves Binary fission.

A cell dividing by binary fission is immortal unless subjected to stress by nutrient depletion or environmental stress.Therefore, a single bacterium continuously divides. 1 cell divides and providing 2 cells and 2 cells divide providing 4 cells and so on.Therefore, the population increases by geometric progression.


REQUIREMENT FOR MICROBIAL GROWTH
PHYSICAL REQUIREMENT
CHEMICAL REQUIREMENT

Requirements for microbial growth

Physical requirements	Chemical requirements
Temperature	Carbon
pH	Nitrogen, Sulfur and Phosphorus
Osmotic pressure	Trace elements
	Oxygen
	Organic growth factor

Physical requirement

<u>OSMOTIC PRESSURE</u>

<u>PH</u>

<u>TEMPERATURE</u>

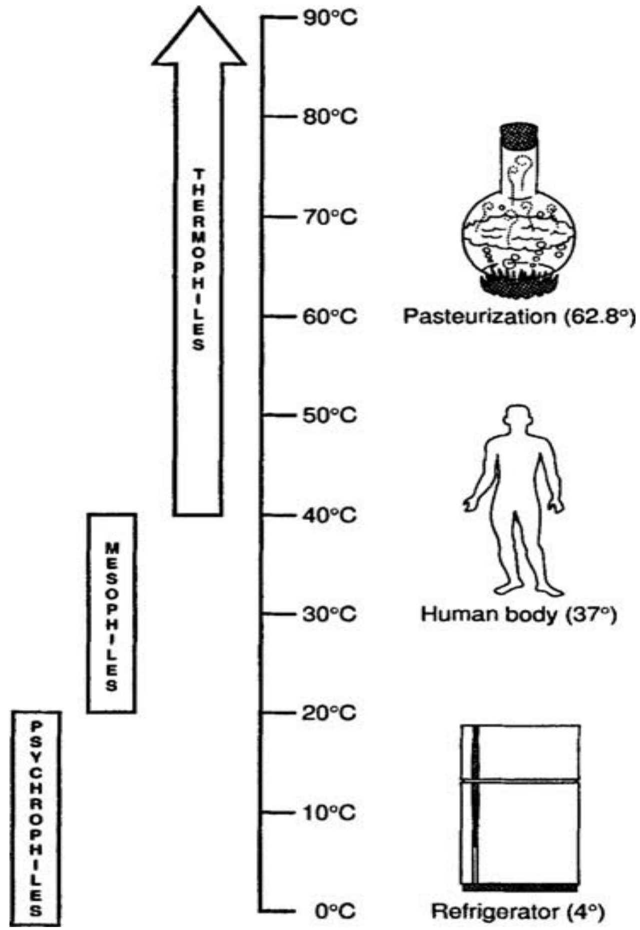
Temperature

- Temperature is the most important factor that determines the rate of growth, multiplication, survival, and death of all living organisms.
- High temperatures damage microbes by denaturing enzymes, transport carriers, and other proteins.
- Microbial membrane are disrupted by temperature extremes.
- At very low temperatures membranes also solidify and enzymes also do not function properly.

THERMOPHILE

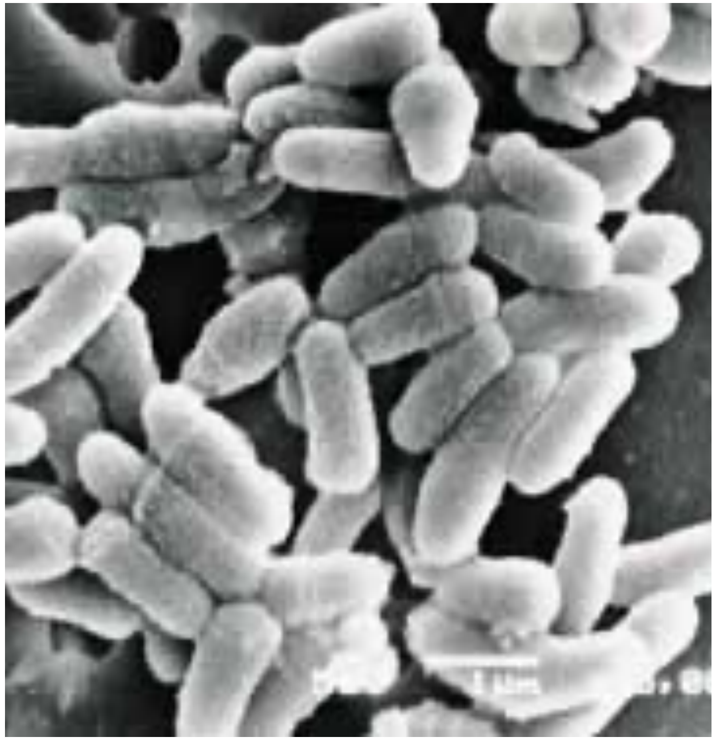
MESOPHILE

PSYCHROPHILE



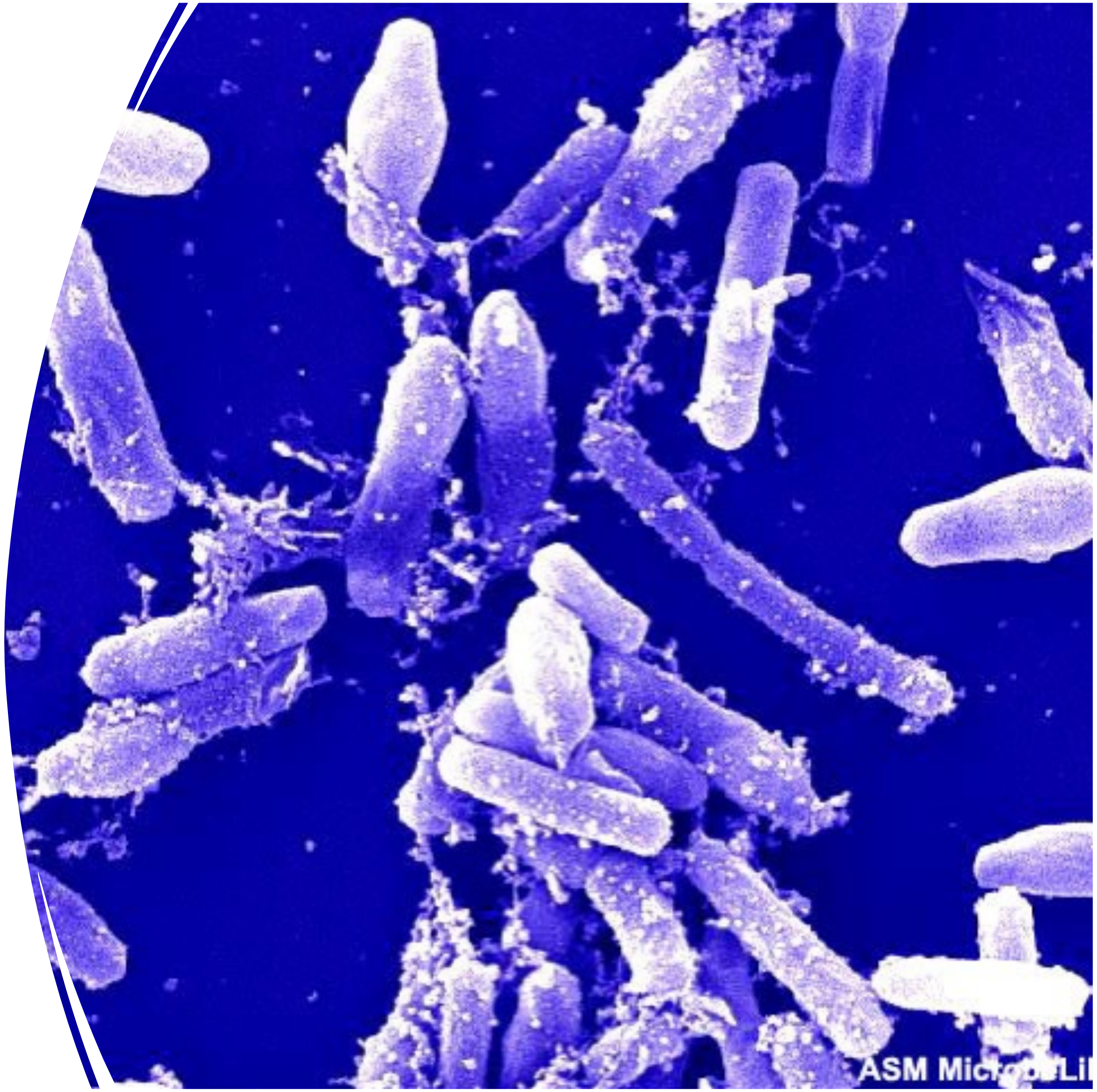
PSYCHROPHILE

- The term *psychrophile* was first used by S. Schmidt-Nelson.
- Extremophilic organisms that are capable of growth and reproduction in cold temperatures
- Temperature range: −20°C to +10°C.
- Examples: *Oscillatoria, Chlamydomonas nivalis, Methanogenium,* etc.



MESOPHILES

- Grows best in moderate temperature.
- Temperature range: 20°C to 45°C.
- Examples: *Escherichia coli, Streptococcus pneumoniae,* etc.

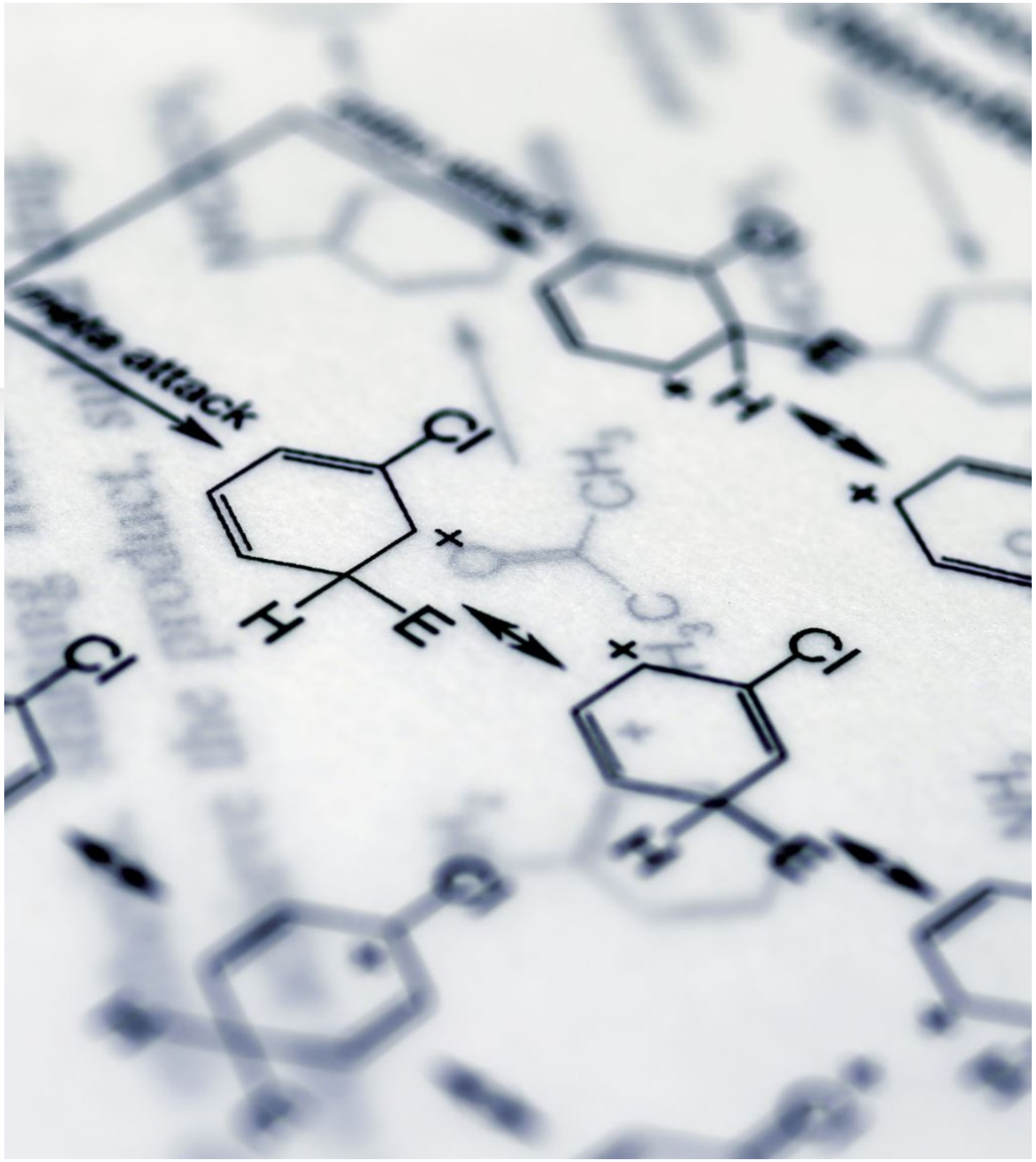


THERMOPHILES

- Derived from Greek word *thermotita* meaning heat and *philia* meaning love.
- Heat-loving microorganisms.
- Grow at 50°C or higher. Their growth minimum is usually around 45°C and often optima between 50 and 80°C.
- Examples: *Thermus aquaticus, Geogemma barossii*, etc.

pH

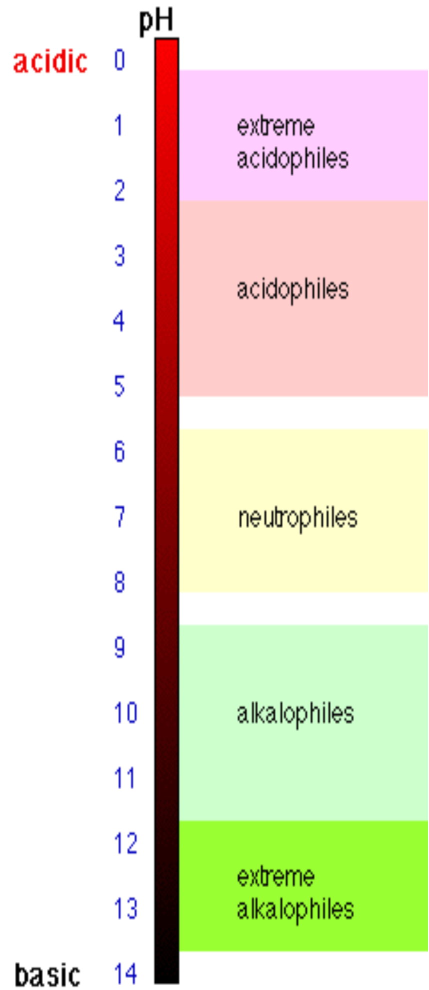
- pH refers to negative logarithm of hydrogen ion concentration.
- Microbial growth is strongly affected by the pH of the medium.
- Drastic variations in cytoplasmic pH disrupt the plasma membrane or inhibit the activity of enzymes and membrane transport protein.



ACIDOPHILES

NEUTOPHILES

ALKALOPHILES



- *ACIDOPHILES*
- Grow between pH 0 and 5.5.
- Examples: *Ferroplasma, Thiobacillus thioxidans, Sulfolobus acidocaldarius,* etc.

- *ALKALOPHILES*
- Grow between pH range of 7.5 to 14.
- Examples*: Thermococcus alcaliphilus,* etc.

- *Neutrophiles*
- Grow between pH 5.5 to 8.0
- Examples: *Lactobacillus acidophillus, E. coli, Pseudomonas aerunginosa,* etc.

Osmotic pressure

- **Osmotic pressure** is the minimum **pressure** which needs to be applied to a solution to prevent the inward flow of water across a SPM.
- Types of solution:
  - 1. Hypotonic 2. Isotonic 3. Hypertonic
- Water activity of a solution is 1/100 the relative humidity of the solution. It is equivalent to the ratio of the vapour pressure of solution to that of pure water.
- **Water activity (a_w)=** $$\frac{\textbf{Vapour pressure of solution}}{\textbf{Vapour pressure of pure water}}$$

OSMOSIS

The selective passage of solvent molecules through a porous membrane from a dilute solution to a more concentrated one.

OSMOTIC PRESSURE

The pressure required to stop osmosis.

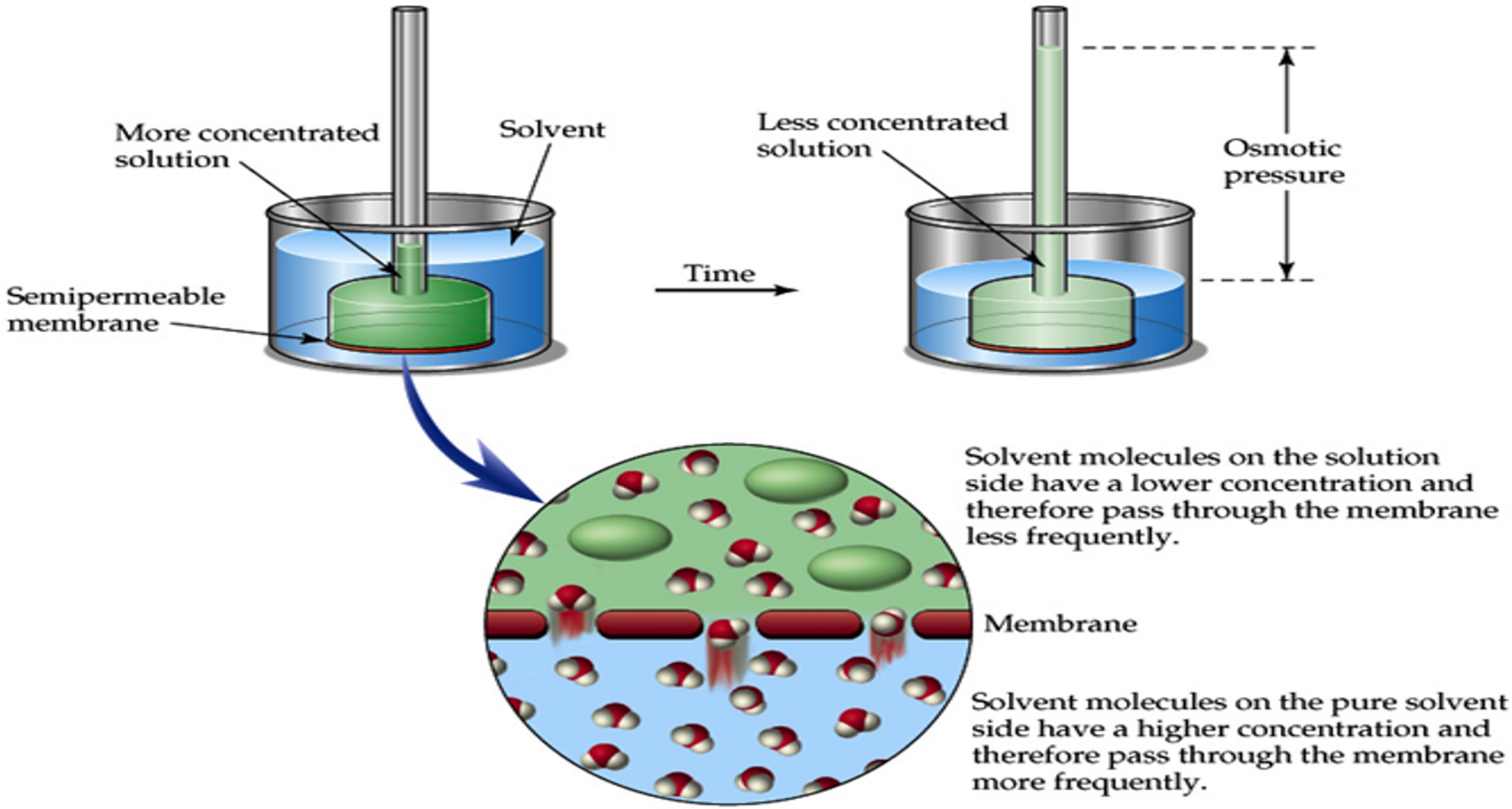
The osmotic pressure of an ideal solution is given by **MORSE EQUATION**

$$\pi = MRT$$

π → OSMOTIC PRESSURE **M → MOLARITY**

R = 0.082 $dm^3 atm mol^{-1} K^{-1}$ **T → absolute temperature**

[redacted]


More concentrated solution
Solvent
Semipermeable membrane
Time
Less concentrated solution
Osmotic pressure
Solvent molecules on the solution side have a lower concentration and therefore pass through the membrane less frequently.
Membrane
Solvent molecules on the pure solvent side have a higher concentration and therefore pass through the membrane more frequently.

Tonicity

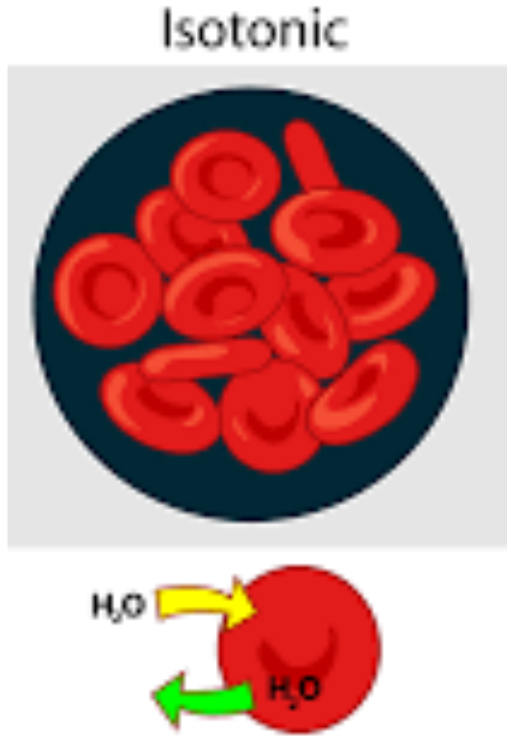
Measure of the osmotic pressure of the two solutions separated by a semipermeable membrane

There are 3 classifications of tonicity:

1. **Isotonic**
2. **Hypertonic**
3. **Hypotonic**

Isotonic

Two solutions which have equal concentration of solutes will have equal osmotic pressure are called isotonic solutions



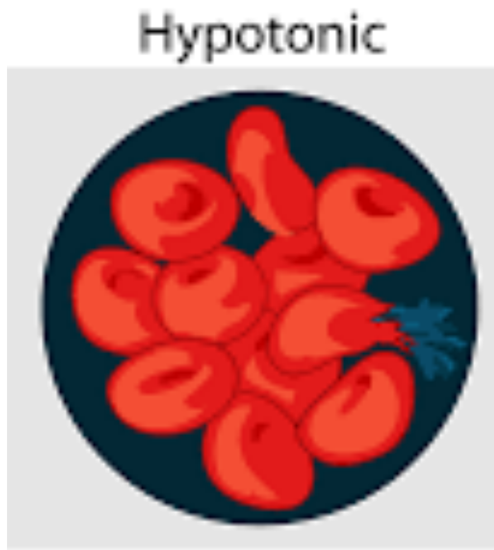
Hypertonic

Solution with a higher concentration of solute is called hypertonic solution

Plasmolysis

Hypotonic

A solution with lower solute concentration is called a Hypotonic solution



Plasmoptysis

Classification of bacteria according to osmotic pressure

1. **Osmotolerant** are those microorganisms which can grow at relatively high salt concentration.

   Examples: *Aeromonas spp., Staphylococcus spp,* etc.

2. **Halophiles**- Grow in the presence of salt at conc. Above 0.2 to 0.6.

   Examples: Halobacterium halobium

CHEMICAL REQUIREMENT

- Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. In the laboratory, bacteria are grown in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth.

- At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the cell's elemental composition, which consists of **C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo**. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in the Table below.

MAJOR ELEMENTAL COMPOSITION OF MICROBIAL CELL

Ele	% DCW	Chemical form used by the microbe	Physiological functions
C	50	Organic compounds, CO, CO_2	major constituents of cell material proteins, nucleic acids, lipids, carbohydrates and others
O	20	Organic compounds, H_2O , O_2, CO_2	
N	14	Organic compounds, NH_4^+, NO_3^-, N2	
H	8	Organic compounds, H2O, H2	
P	3	HPO_4^{2-}	nucleic acids, phospholipids, teichoic acid, coenzymes
S	1	organic sulfur compounds, SO_4^{2-}, HS^-, S^0, $S_2O_3^{2-}$	proteins, coenzymes
k	1	K^+	major inorganic cation, compatible solute, enzyme cofactor
Na	1	Na^+	transport, energy transduction
Ca	0.5	Ca^{2+}	enzyme cofactor, bound to cell wall
Mg	0.5	Mg^{2+}	enzyme cofactor, bound to cell wall, membrane & phosphate esters
Cl	0.5	Cl^-	major inorganic anion
Fe	0.2	Fe^{2+}, Fe^{3+}	cytochromes, ferredoxin, cofactor

Carbon

Structural backbone of living matter, it is needed for all organic compounds to make up a living cell

Chemoheterotrophs get most of their carbon from the source of their energy---organic materials such as proteins, carbohydrates and lipids

Chemoautotrophs and **photoautotrophs** derive their carbon from carbon dioxide

Nitrogen, Sulfur and Phosphorus

For synthesis of cellular material

Nitrogen and sulfur is needed for protein synthesis

Nitrogen and phosphorus is needed for syntheses of DNA, RNA and ATP

Nitrogen- 14% dry weight of a bacterial cell

Sulfur and phosphorus- 4%

Trace Elements

- Microbes require very small amounts of other mineral elements, such as Fe, Cu, Mo, Zn
- Essential for certain functions of certain enzymes
- Assumed to be naturally present in tap water and other components of media

TRACES OF NUTRITION'S

Element	Chemical form used by the microbe	Physiological functions
Mn	Mn^{2+}	superoxide dismutase, photosystem II
Co	Co^{2+}	coenzyme B12
Ni	Ni^{+}	hydrogenase, urease
Cu	Cu^{2+}	cytochrome oxidase, oxygenase
Zn	Zn^{2+}	alcohol dehydrogenase, aldolase, alkaline phosphatase, RNA and DNA polymerase, arsenate reductase
Se	$SeO3^{2-}$	formate dehydrogenase, glycine reductase
Mo	$MoO4^{2-}$	nitrogenase, nitrate reductase, formate dehydrogenase, arsenate reductase
W (tungsten)	$WO4^{2-}$	formate dehydrogenase, aldehyde oxidoreductase

Oxygen

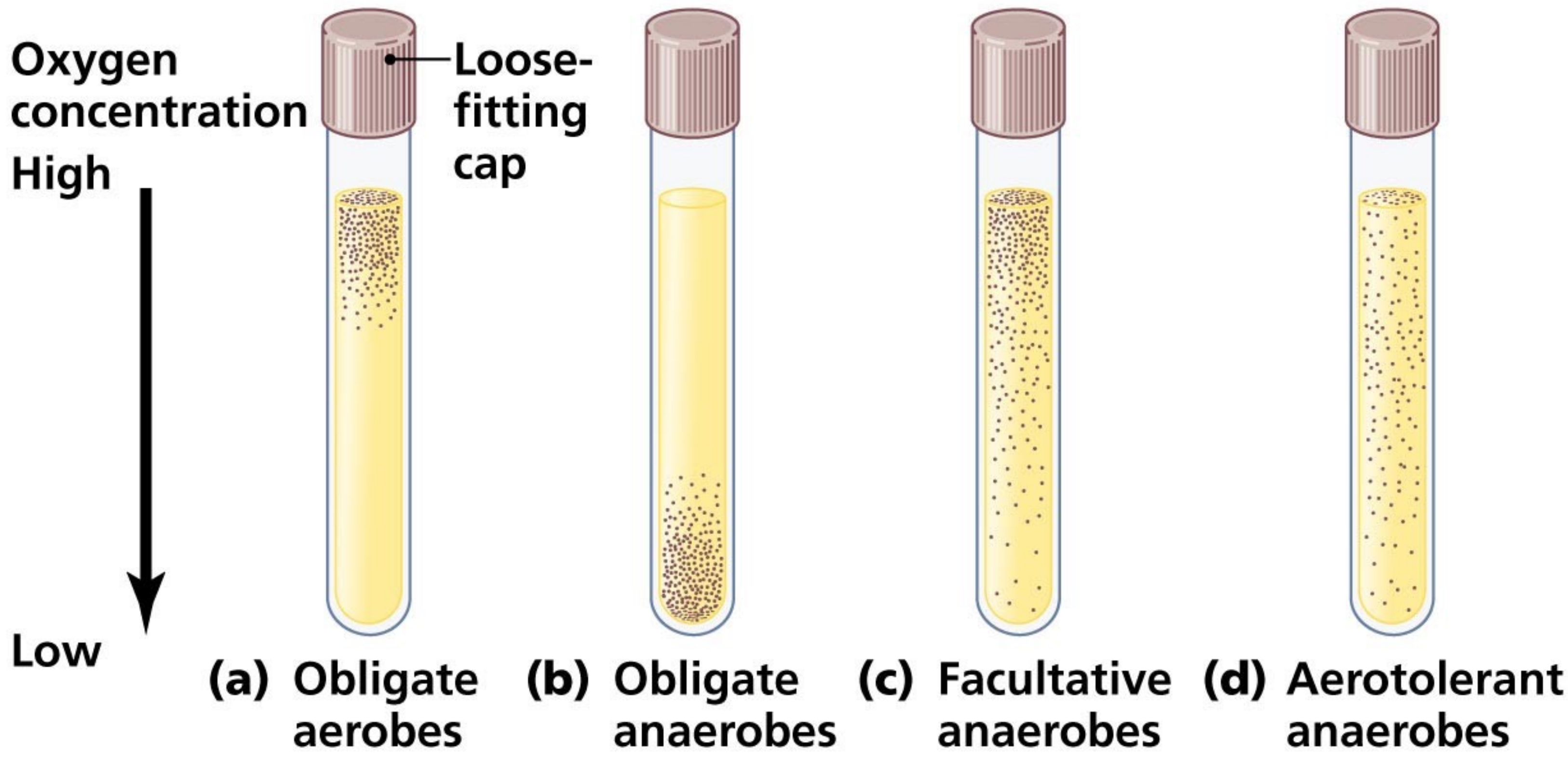
Obligate Aerobes - only aerobic growth, oxygen required, growth occurs with high concentration of oxygen

Facultative Aerobes - both aerobic and anaerobic growth, greater growth in presence of water, growth is best in presence of water but still grows without presence of oxygen

- **Obligate Anaerobe** - only anaerobic growth, growth ceases in presence of oxygen, growth occurs only when there is no oxygen

- **Aerotolerate Anaerobe** - only anaerobic growth, but continues in presence of oxygen, oxygen has no effect

- **Microaerophiles** - only aerobic growth, oxygen required in low concentration, growth occurs only where a low concentration of oxygen has diffused into medium


Oxygen
concentration
High
Low
Loose-
fitting
cap
(a) Obligate aerobes
(b) Obligate anaerobes
(c) Facultative anaerobes
(d) Aerotolerant anaerobes

Organic Growth Factors

Essential organic compounds an organism is unable to synthesize, they must be directly obtained from the environment

Growth factors are organized into three categories.

- **1.purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)
- **2.amino acids**: required for the synthesis of proteins
- **3.vitamins**: needed as coenzymes and functional groups of certain enzymes

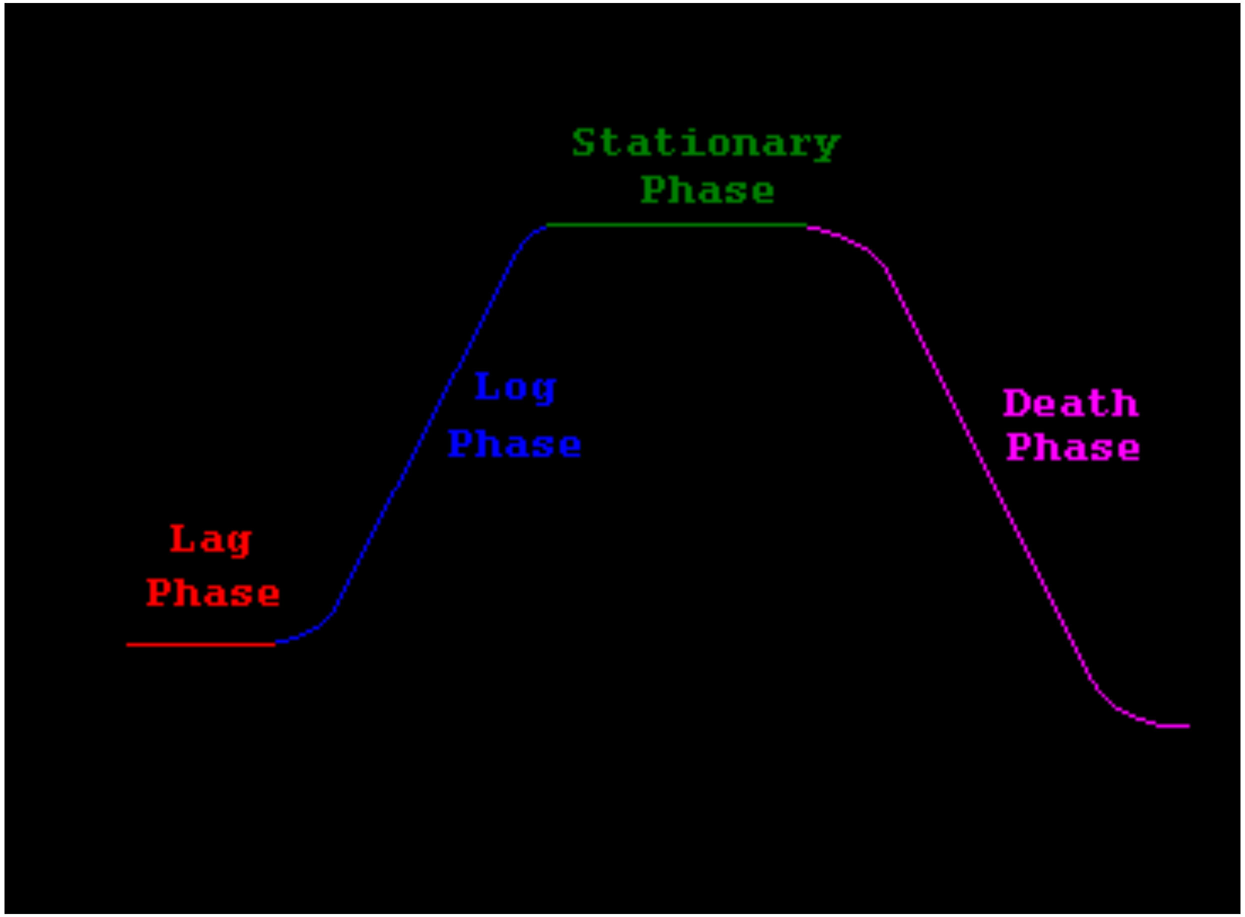
Some bacteria lack the enzymes needed for synthesis for certain vitamins, so they must obtain them directly

Examples: amino acids, purines, pyrimidines

Bacterial Growth Curve

The growth of bacteria in batch culture can be modeled with 4 different phases:

- **1.Lag phase**
- **2.Log phase** or **exponential phase**
- **3.Stationary phase**
- **4.Death phase**



LAG PHASE

- Period of little or no cell division
- Can last for 1 hour or several days
- Cells are not dormant
- Undergoing a period of intense metabolic activity : DNA and enzyme synthesis

LOG PHASE

- Period of growth also known as logarithmic increase
- Sometimes called as exponential growth phase
- Cellular respiration is most active during this period
- Metabolic activity is active and is most preferable for industrial purposes
- Sensitive to adverse conditions

STATIONARY PHASE

- Period of equilibrium
- Metabolic activity of surviving cells slows down which stabilizes the population
- Cause of discontinuity of exponential growth is not always clear
- May play a role: exhaustion of nutrients, accumulation of waste products and harmful changes in pH
- **Chemostat** – continuous culture used in industrial fermentation

DEATH PHASE

- Also known as **Logarithmic Decline Phase**
- Continues until a small fraction of the population is diminished
- Some population dies out completely
- Others retain surviving cells indefinitely while others only retain for a few days

THANK YOU