Microbial Growth & Environmental Factors Affecting Growth

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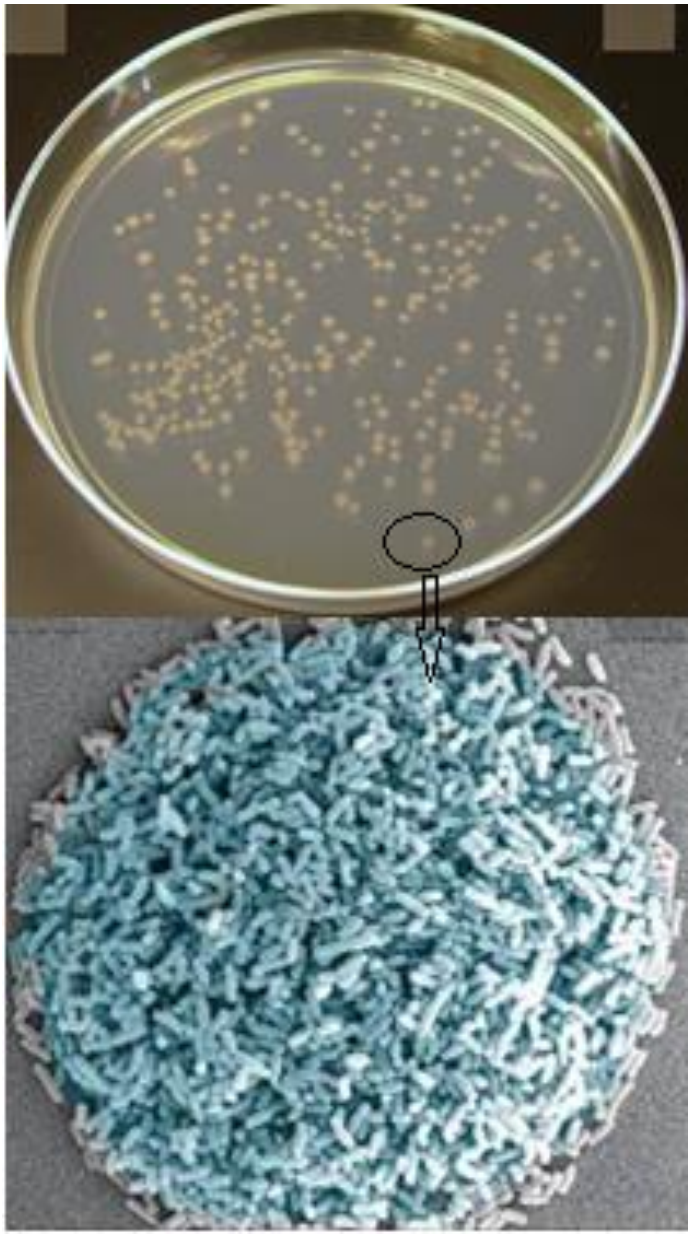
Microbial Growth

Refers to an increase in **cell number**, not in cell size.

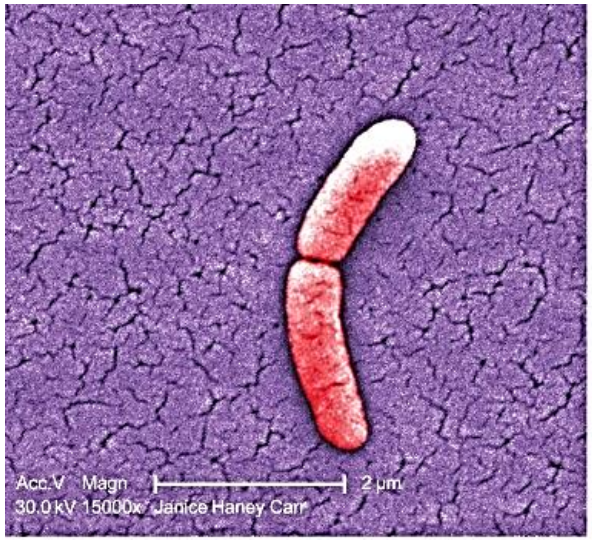
Bacteria grow on solid media as **colonies**. A **colony** is defined as a visible mass of microorganisms all originating from a single mother cell

Bacteria grow and divide by **binary fission**, a rapid and relatively simple process.

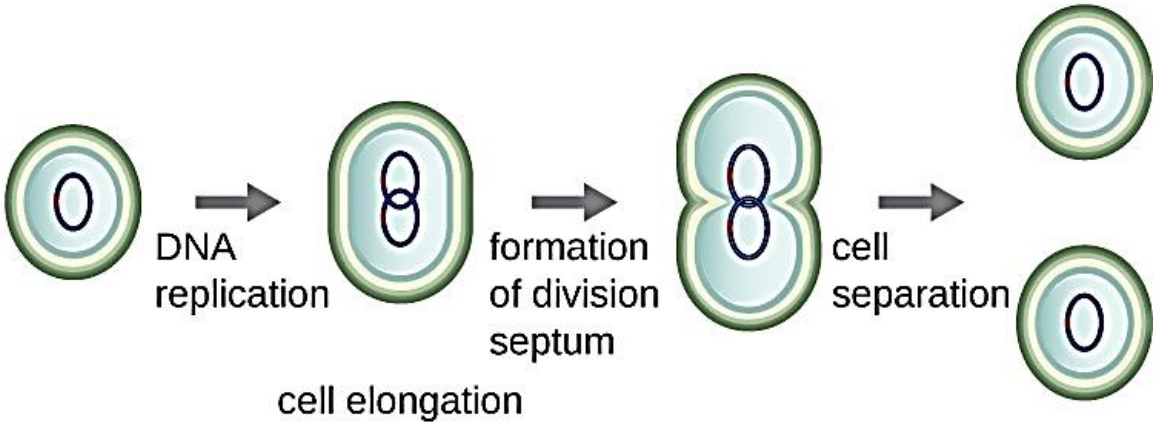


Bacterial Growth: Binary Fission

Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome.



(a)



(b)

Generation Time: Time required for a cell to divide, and its population to double.

Generation time varies considerably:

E. coli divides every 20 minutes.

Most bacteria divide every 1 to 3 hours.

Some bacteria require over 24 hours to divide.

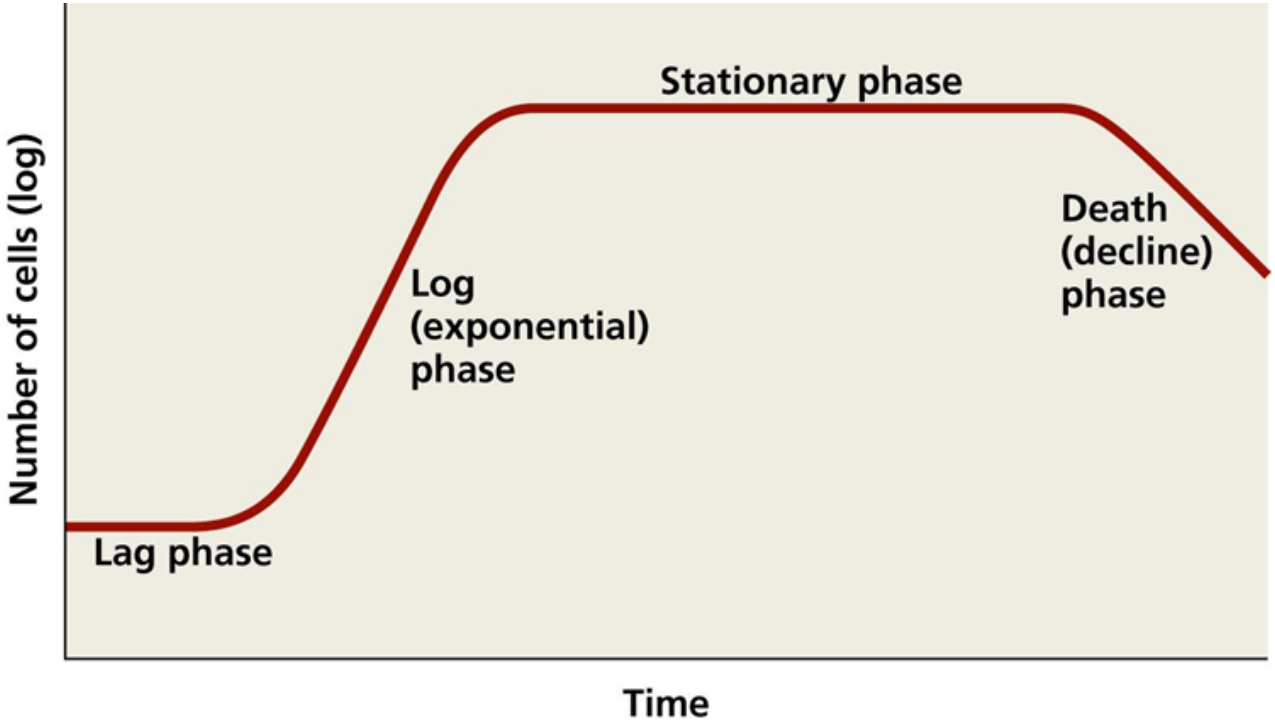
Phases of Growth

1.Lag phase: During this phase, metabolic activity occurs but cells do not divide. This can last a few minutes up to many hours.

2.Log phase(logarithmic or exponential): The cells start dividing & their number increase with time.

3.Stationary phase: The rate of multiplication & death becomes almost equal, because of depletion of nutrient & accumulation of toxic product .

4.Decline phase: In this phase population decreases due to death of cells.



Measuring Microbial Growth

Direct Methods of Measurement

1. Plate count

2. Filtration

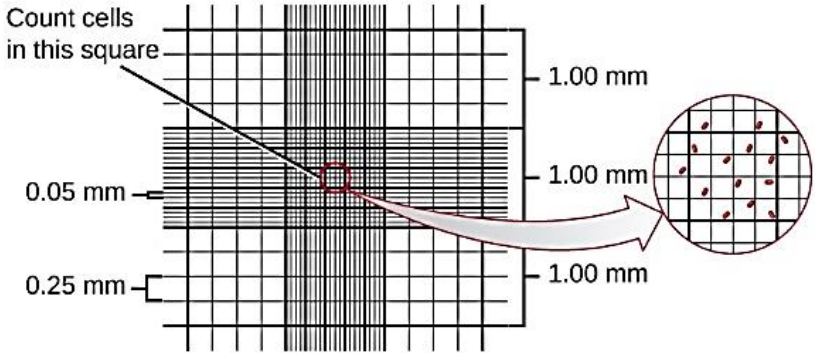
-Used to measure small quantities of bacteria.

- **Example**: Fecal bacteria in a lake or in ocean water.

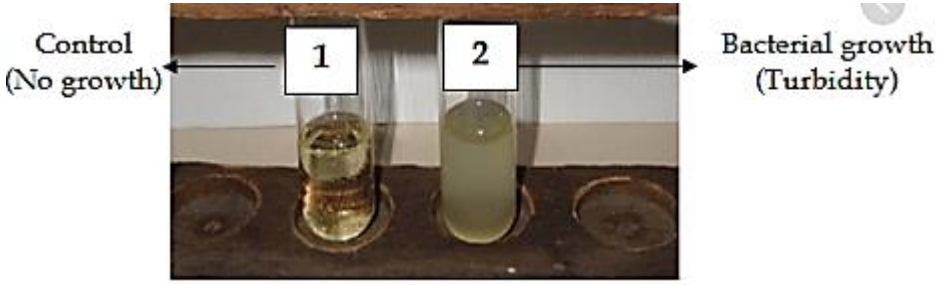
A large sample (100 ml or more) is filtered to retain bacteria.
Filter is transferred onto a Petri dish.
Incubate and count colonies.

3- Direct Microscopic Count

Involves transferring a known volume of a culture to a calibrated slide and counting the cells under a light microscope. The calibrated slide is called a Petroff-Hausser chamber



Measuring Microbial Growth



Indirect Methods of Measurement

1-Turbidity: As bacteria multiply in media, it becomes turbid.

Spectrophotometer is used to measure turbidity

2. Metabolic Activity

-As bacteria multiply in media, they produce certain products such as Carbon dioxide and Acids.

-Measure metabolic products.

3. Dry Weight

(Biomass concentration :It is the dry weight of cells Per unit volume of culture).

-Bacteria in liquid media are centrifuged.

- Resulting cell pellet is weighed.

1. Plate count:

Most frequently used method of measuring bacterial populations.

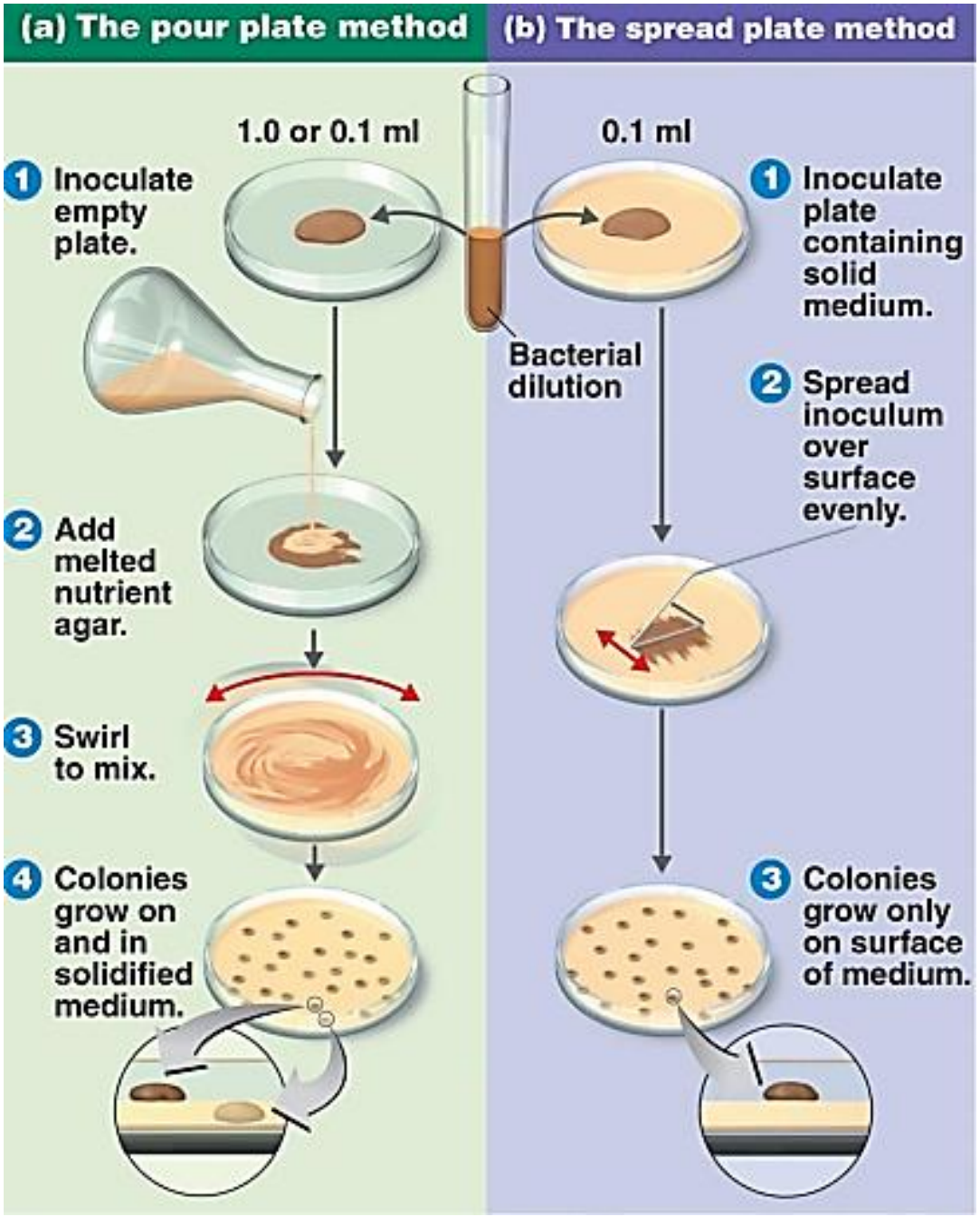
Inoculate plate with a sample and count number of colonies

A-Pour Plate: In this method, fixed amount of inoculum from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

B. Spread Plate:

Introduce an inoculum onto the **surface** of Petri dish

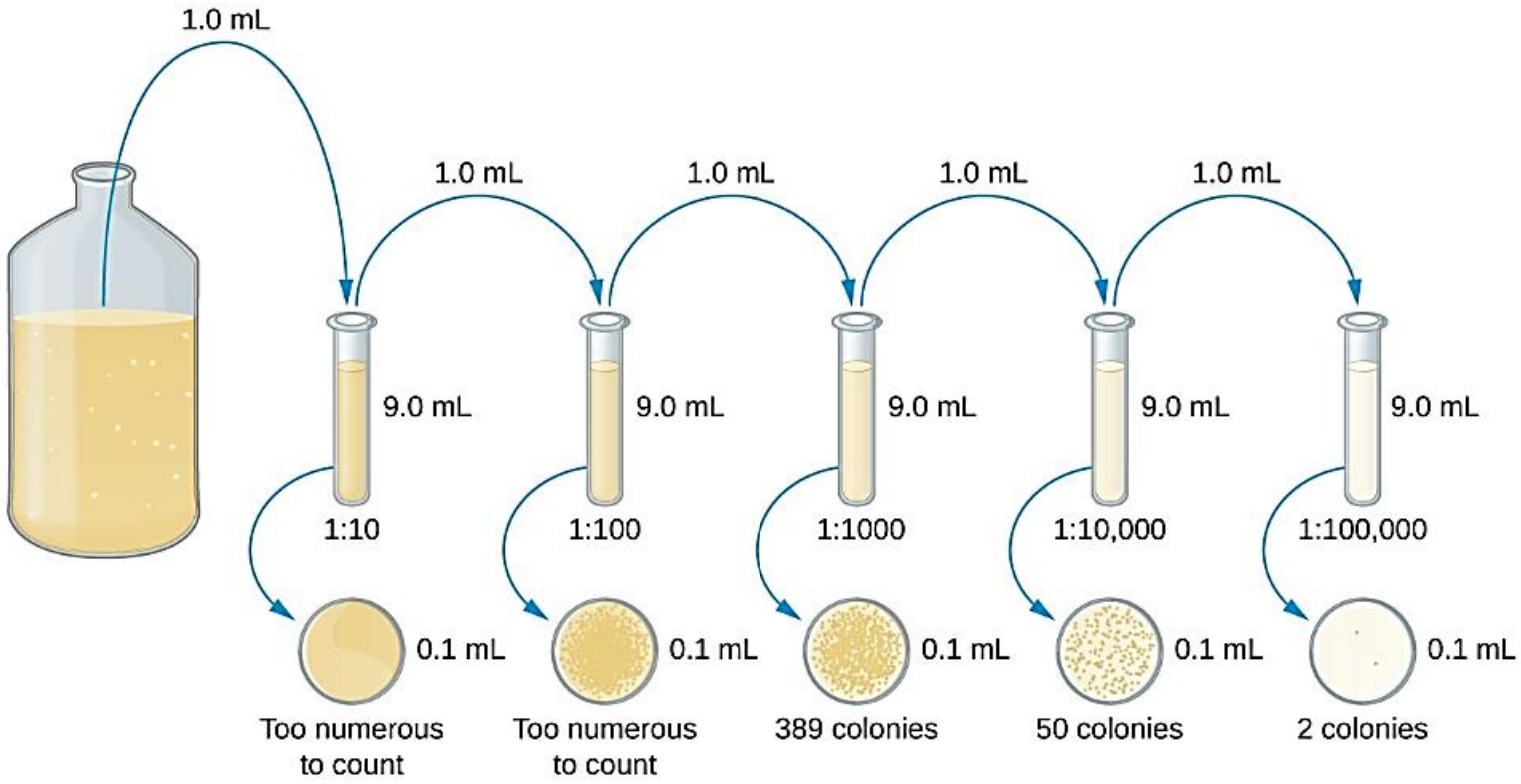
-Microbiologists typically count plates with 30–300 colonies. Samples with too few colonies (<30) do not give statistically reliable numbers, and overcrowded plates (>300 colonies) make it difficult to accurately count individual colonies.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate.
1 Inoculate plate containing solid medium.
Bacterial dilution
2 Add melted nutrient agar.
2 Spread inoculum over surface evenly.
3 Swirl to mix.
4 Colonies grow on and in solidified medium.
3 Colonies grow only on surface of medium.

Serial Dilution

The serial dilution of a culture is an important first step before proceeding to either the pour plate or spread plate method. The goal of the serial dilution process is to obtain plates with CFUs in the range of 30–300, and the process usually involves several dilutions in multiples of 10 to simplify calculation.

Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor.



Calculating the number of bacteria per mL of serially diluted bacteria:

To calculate the number of bacteria per mL of diluted sample one should use the following equation:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}}$$

For example, if for the 1x 10^{-8} dilution plate you plated **0.1 mL** of the diluted cell suspension and counted 200 bacteria, then the calculation would be:

200/0.1 mL x 10^{-8} or 200/10^{-9} or 2.0 x 10^{11} bacteria per mL.

Growth Requirements

Physical Requirements

1.Temperature: Microbes can be roughly classified according to the range of temperature at which they can grow.

1.Psychrophiles(0-20°C)
2-Psychrotrophs (4-25°C).
3.Mesophiles(20-45°C)
4.Thermophiles(50-80°C)
5.Hyperthermophiles(80-110°C)

2-Hydrogen ion concentration(pH)

Organisms can be classified as:

A. Acidophiles: .

Grow at very low pH (0.1 to 5.4)

B. Neutrophiles:

Grow at pH 5.4 to 8.5.

C. Alkaliphiles:

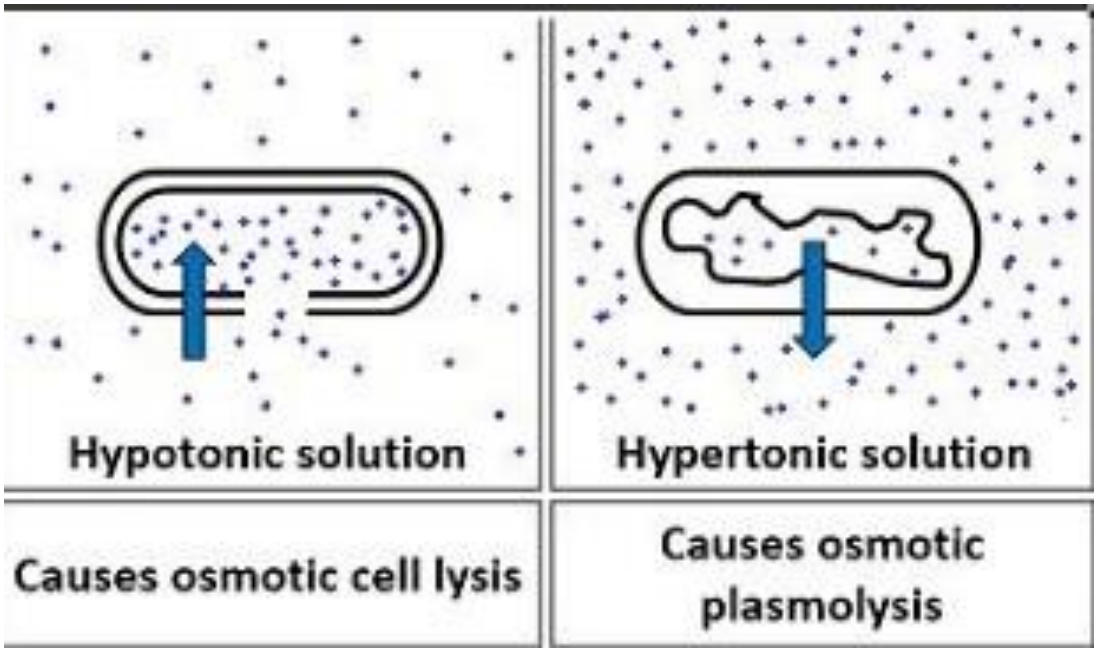
Grow at alkaline or high pH (7 to 12 or higher).

Osmotic Pressure :

Cells are 80 to 90% water.

A. Hypertonic solutions: High osmotic pressure removes water from cell, causing shrinkage of cell membrane (plasmolysis).

B. Hypotonic solutions: Low osmotic pressure causes water to enter the cell. In most cases cell wall prevents excessive entry of water.



3- Osmotic Pressure :

- **Halophiles**: Require moderate to large salt concentrations. Ocean water contains 3.5% salt. Most bacteria in oceans.
- **Extreme or Obligate Halophiles**: Require very high salt concentrations (20 to 30%). Bacteria in Dead Sea.
- **Facultative Halophiles**: Do not require high salt concentrations for growth, but tolerate 2% salt or more.

Growth Requirements

Chemical Requirements

1-Oxygen: Organisms that use molecular oxygen (O2), produce more energy from nutrients than anaerobes.

Can classify microorganism based on their oxygen requirements:

A. Obligate Aerobes: Require oxygen to live.

Example: *Pseudomonas*.

B. Facultative Anaerobes: Can use oxygen, but can grow in its absence. Have complex set of enzymes.

Examples: *E. coli* and *Staphylococcus*,.

C. Obligate Anaerobes: Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.

Examples: *Clostridium* bacteria that cause tetanus and botulism

D. Aerotolerant Anaerobes: Can't use oxygen, but tolerate its presence. Can break down toxic forms of oxygen.

Example: *Lactobacillus*.

E. Microaerophiles: Require oxygen, but at low concentrations. Sensitive to toxic forms of oxygen.

Example: *Campylobacter*

Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles

2. Carbon: Makes up 50% of dry weight of cell. Structural backbone of all organic compounds.
- **Chemoheterotrophs**: Obtain carbon from their energy source: lipids, proteins, and carbohydrates.
-**Chemoautotrophs and Photoautotrophs** : Obtain carbon from carbon dioxide.

3. Nitrogen, Sulfur, and Phosphorus: .

A. Nitrogen: Makes up 14% of dry cell weight. Used to form amino acids, DNA, and RNA.

Sources of nitrogen:

Nitrates,Nitrogen gas (N2),Protein and Ammonium,

B. Sulfur: Used to form proteins

Sources of sulfur

.- Protein,Hydrogen sulfide and sulfates

C. Phosphorus: Used to form DNA, RNA, ATP, and phospholipids .

Sources: Mainly inorganic phosphate salts and buffers.

4. Other Elements: Potassium, magnesium, and calcium are often required as enzyme cofactors.

5-Trace Elements:

Many are used as enzyme cofactors.

Iron,Copper and Zinc

Thanks