Practical Principles of Microbial Physiology // 4th Stage **Lab 5**
Effect of osmotic pressure on microbial growth **3 pages**

The growth of bacteria profoundly affects by the availability of water in an environment which decrease with increase in concentration of solutes such as salts, this result water becomes involved in breaking ionic bonds and forming salvation shells around charged species to maintain them in solution.

Osmosis: is the diffusion of water from area of low solutes concentration "where water is more plentiful" to area of high solutes concentration "where water is less".

Osmotic pressure: is the force developed when two solutions of different solute concentrations are separated by a membrane that is permeable only to the solvents. The solvent is the liquid, usually water, that dissolves a substance (the solute).

Water activity (a_w): is a term describing the availability of water to microorganisms. a_w can be decrease by the addition of any soluble molecules such as salt (NaCl) and sugar.
Pure water has an a_w of 1.00, **most** bacteria fail to grow in a food or other media where the a_w is lower than **0.94**. Certain molds and bacteria can grow on fish immersed in saturated salt solution where the a_w is about **0.75**. Some molds can grow in foods with a_w **0.62 - 0.65**.

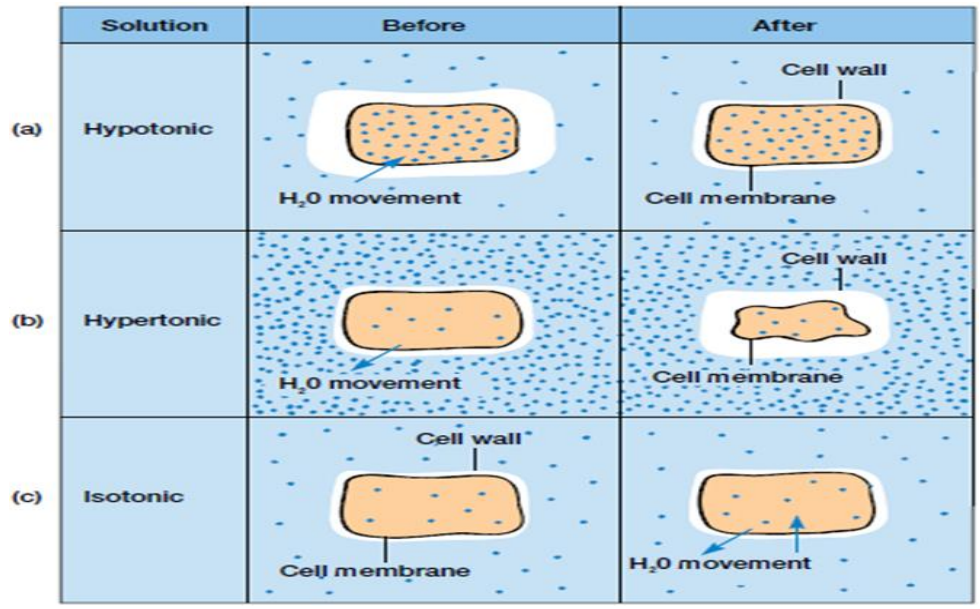
Effects of low water levels

- Impaired transport of nutrients required in metabolism
- Inhibit enzymes activity.
- Loss of membrane fluidity.
- Longer lag phase and slower growth.

According to the concentration of solutes there are three different media

1. **Hypotonic media:**
   - External concentration of solutes is lower than cell's internal environment.
   - water diffuses to the cytoplasm.
   - Cells swell and may be burst
2. **Hypertonic media:**
   - Environment has higher solute concentration than cell's internal environment.
   - Water diffuses out from the cytoplasm.
   - Cells undergo **plasmolysis** resulting in a loss of water, dehydration of the cytoplasm and shrinkage of cell membrane away from the cell wall.

3. **Isotonic media:**
   - External concentration of solutes is equal to cell's internal environment
   - Diffusion of water equal in both directions
   - No net change in cell volume.



<u>Microorganisms can be grouped based on their ability to grow in different concentration of solutes</u>

- **Non-halophiles**: Microbes that require a highwater activity (near or at 1.00) e.g., *Escherichia coli.*
- **Halophiles**: microorganisms which require high concentration of NaCl to grow, depending on their salt requirements halophiles are divided into
  - Mild halophiles (1 - 6 % NaCl) e.g., *Methanosalsus* and *Chlorobium limicola*
  - Moderate halophiles (6 - 15% NaCl) e.g., *Aliivibrio fischeri.*
  - Extreme halophiles (15 - 30% NaCl) e.g., *Halobacterium salinarium.*
- **Halotolerant**: refers to the ability of a non-halophile to grow in moderate concentration of salt like *Staphylococcus aureus* can tolerate NaCl concentration that reach to 11%.
- **Osmophiles**: microorganisms able to grow in environments high in sugar as a solute, e.g., *Saccharomyces bailii.*

Aim:

To examine the effect of various salt concentrations on the growth of bacterial species, bacterial tolerance to NaCl can be observed by the amount or lack of bacterial growth.

Materials:

- Nutrient agar media containing (0.8, 5, 7, 9, 11) % NaCl
- Broth cultures of unknown bacterial samples.
- Micropipette
- Swabs

Procedure

1. With wax pencil, label each nutrient agar plate (has specific NaCl concentration) with bacterial sample no and date.
2. Inoculate plate of each of these nutrient agar media with appropriate microorganism.
3. Incubate all the plates inverted at 37± 2° C for 24 - 48 hours.
4. After incubation time, observe the effect of osmotic pressure on these bacteria by the presence or absence of growth in each plate. Record this growth as – (no growth) and + (bacterial growth)

Results

Observations and Interpretations

Microorganisms	**NaCl Concentration %**					**Classification**
	0.8	**5**	**7**	**9**	**11**	
Bacterial Sample 1						
Bacterial Sample 2						
Bacterial Sample 3						
Bacterial Sample 4						