Microbiology Lab
Microbial Growth: Environmental Factors

Ex. 2-7: Oxygen Requirements: Effects on Growth
A. Bacteria may use oxygen as part of the cellular respiration. **Cellular respiration** is the process of oxidizing glucose (releasing energy stored in its chemical bonds) to produce ATP (the energy source the cell uses). Cellular respiration may occur as follows:
   1. **Glycolysis**: Glucose (6 C) → 2 Pyruvic acid (3 C) + 2 ATP
   a. **Fermentation**: Reset the electron carriers for the next glycolysis reaction. Examples include:
      i. In yeast, pyruvic acid → alcohol
      ii. In muscles, pyruvic acid → lactic acid
   b. With **Anaerobic Respiration**, you only yield 2 ATP from glycolysis.
   2. **Aerobic Respiration** – completes the oxidation of glucose to yield a lot more ATP, but requires O₂ as the terminal electron acceptor.
      a. **Transition step**: Pyruvic acid (3 C) → Acetyl Group (2 C) + CO₂
         i. Bind the Acetyl Group to a carrier molecule, Co Enzyme A, producing Acetyl CoA
      b. **TCA Cycle**: Finalize the oxidation of glucose to CO₂, yielding a lot of reducing power
      c. **Electron Transport Chain and Proton Motive Force**: Use reducing power to make 34 ATP.
B. Oxygen can be deadly to bacteria because it forms superoxides like hydrogen peroxide. To survive in an oxygen rich environment, bacteria must have enzymes such as **oxygen dismutase** or **catalase** to neutralize those super oxides.
C. Bacteria can be classified into 5 categories based upon their O₂ requirements.

<table>
<thead>
<tr>
<th>O₂ requirements</th>
<th>Obligate aerobe</th>
<th>Obligate anaerobe</th>
<th>Facultative anaerobe</th>
<th>Aerotolerant anaerobe</th>
<th>Microaerophile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of oxygen on growth</td>
<td>Oxygen required. Dies without oxygen.</td>
<td>Dies if oxygen is present</td>
<td>Grows better in the presence of oxygen, but can survive without it.</td>
<td>Indifferent to the presence or absence of oxygen.</td>
<td>Low oxygen concentration is needed for growth.</td>
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<tr>
<td>Enzyme Presence or Absence</td>
<td>Catalase and Superoxide Dismutase (SOD) neutralize the toxic forms of oxygen.</td>
<td>NO enzymes to neutralize toxic oxygen.</td>
<td>Catalase and Superoxide Dismutase (SOD) neutralize the toxic forms of oxygen.</td>
<td>SOD present to partially neutralize toxic oxygen.</td>
<td></td>
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<tr>
<td>Example Organisms</td>
<td>Mycobacterium, Human beings</td>
<td>Clostridium</td>
<td>Streptococcus, Staphylococcus, Enterobactericeae</td>
<td>Lactobacillus</td>
<td>Neisseria gonorrhoeae</td>
</tr>
</tbody>
</table>
D. We can go a long way toward determining the oxygen requirements of our bacteria utilizing a stab agar culture.

Ex. 2-8: Temperature: Effects on Growth
A. **Enzymes** (catalysts for metabolic reactions) have a minimum, optimum, and maximum temperature for activity.
   1. Maximum enzyme activity will occur at the optimum temperature.
   2. At 10°C increase will speed up the metabolic reactions (run by enzymes) by double.
   3. At 10°C decrease will half the metabolic reaction rate.
B. Bacteria can be classified into 4 main groups
   1. **Psychrophile** – love the cold, below 20°C
   2. **Mesophile** – love middle temperatures (15-45°C). Human body temperature is just below 37°C.
      a. Most bacteria that cause disease in humans are mesophiles.
   3. **Thermophile** – love the heat, above 40°C
   4. **Hyperthermophile** – LOVE the heat, above 80°C
Ex. 2-9: pH: Effects on Growth

The concentration of hydrogen ions is commonly expressed in terms of the pH scale.

- Low pH corresponds to high hydrogen ion concentration.
  - We call such solutions acidic.
- High pH corresponds to low hydrogen ion concentration.
  - We call such solutions basic or alkaline.
- The pH scale is a negative logarithmic scale with a base number of ten.
  - Each decrease in pH by one pH unit means a tenfold increase in the concentration of hydrogen ions.
  - Thus, a solution with a pH of 2 is 10 times more acidic than a solution with pH of 3.
  - How much stronger of a base is a solution with pH of 10.3 than a solution with pH of 8.3?

- Some substances enable solutions to resist pH changes when an acid or base is added. Such substances are called buffers.
  - Buffers are very important in helping organisms maintain a relatively constant pH.

Below is a pH chart that will give you some understanding of pH values. (You are NOT expected to memorize these pH values.)

The hydrogen ion concentration affects proteins and other charged molecules in the cell. Based on their temperature requirements, bacteria are divided into four groups...

- Most bacteria are neutrophiles, which live within the pH range of 5.5 to 8.5.
  - They love it around neutral pH = 7
- Acidophiles grow optimally at a pH below 5.5.
  - They love an acidic pH
- Alkaliphiles grow optimally at a pH above 8.5.
  - They love a basic pH
- Which of these is most likely to cause disease in humans?
  - Exception of H. pylori (an acidophile) growing in stomach (pH of 1-3).

Ex. 2-10: Osmotic Pressure and Microbial Growth

The availability of water in an environment can profoundly affect the growth of bacteria.

Osmosis – the diffusion of water from an area of high concentration, across a semi-permeable membrane, to an area of low concentration.

Microorganisms can be grouped based upon their ability to cope with extreme osmotic pressure into the following groups...

- Halophiles – require high concentrations of salt to grow. They are found in salt lakes.
- Halotolerant – capable of growing in moderate concentrations of salt.
- Osmophiles – able to grow in environments where sugar concentrations are excessive.
  - These organisms can ruin maple syrup, jams, and jellies. They are typically fungi (such as yeast).
Cells always exist in a solution that contains water. **Water is the solvent, and anything dissolved in that water is the solute.** As the solute concentration in a solution increases, the water concentration decreases & vice versa. The average cell is about 75% water. **Only the water will move in & out of the cell** as the solutes cannot pass the plasma membrane.

![Diagram of cell concentration](image)

There are 3 different solutions a cell can exist in:

1. **Hypotonic solutions** have less solutes than the cell. Therefore, they have more water than the cell.
   
   a. We’re going to submerge our cells in a solution that is 0% solutes (100% water).

   ![Hypotonic solution](image)

   **Results**
   
   This cell will swell and pop. Death by lysis.

2. **Isotonic solutions** have the same concentration of solutes as the cell. Therefore, these solutions have the same concentration of water as the cell.
   
   a. Because our cells are 25% solutes (75% water), an isotonic solution must be 25% solutes (75% water).

   ![Isotonic solution](image)

   **Results**
   
   These cells remain perfectly healthy.

3. **Hypertonic solutions** have more solutes than the cell. Therefore, these solutions have less water than the cell.
   
   a. We’re going to submerge our cells in a solution that is 40% solutes (60% water).

   ![Hypertonic solution](image)
60% water

75% water
Bacterial Cell

75% water
Animal Cell

Results
These cells will dehydrate.

Bacterial Cell
Animal Cell

Both cells die by plasmolysis.