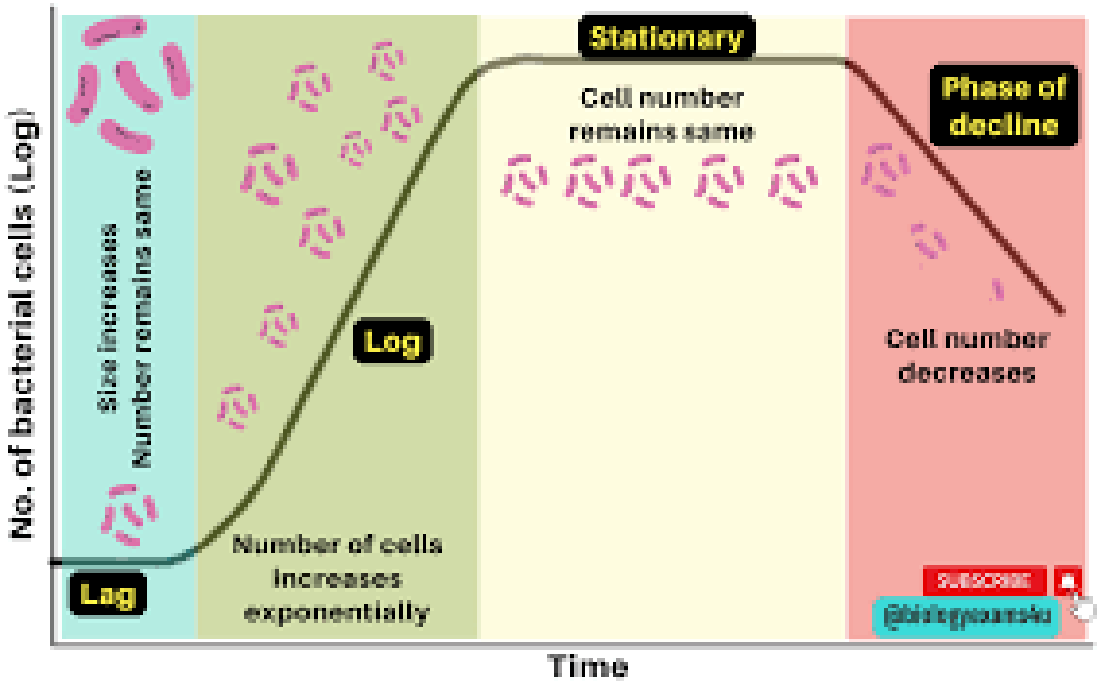
GOVERNMENT COLLEGE (A) RAJAHMUNDRY
DEPARTMENT OF MICROBIOLOGY
SECOND YEAR
IV SEMESTER
COURSE 10



MICROBIAL PHYSIOLOGY AND METABOLISM

IV SEMESTER
COURSE 10: - MICROBIAL PHYSIOLOGY AND METABOLISM

CREDITS -_3

UNIT I: Microbial Nutrition - No. of hours: 9

1. Nutritional requirements of Microorganisms
2. Methods of uptake of nutrients by cells- Primary and secondary active transport, concept of uniport, symport and antiport Group translocation; Iron uptake
3. Nutritional groups of microorganisms-based on C, energy and electron. sources
4. Growth media - synthetic, nonsynthetic, selective, enrichment and differential media.

UNIT II: Microbial Growth ¯No. of hours:9

1. Microbial Growth- Definitions of growth, generation time and specific growth rate; different phases of growth in batch cultures;
2. Synchronous, continuous, biphasic growth.
3. Factors influencing microbial growth.
4. Methods for measuring microbial growth - Direct microscopy, viable count estimates, turbidometry & biomass.

UNIT III: Thermodynamics; Breakdown of Carbohydrates . No.of hrs: 9

1. Thermodynamics in biological systems - Concept of free energy, Enthalpy, Standard Free Energy change of reaction, Entropy. First and Second law of Thermodynamics. Open and Closed system.
2. Structure and properties of ATP, Standard Free energy change of hydrolysis of ATP and other high energy compounds. Biological oxidation-reduction reactions. Structure and Function of NAD and FAD.
3. Breakdown of carbohydrates· Glycolytic pathways- EMP, HMP shunt/pentose phosphate pathway and ED; TCA cycle.

UNIT IV: Microbial Respiration and Fermentation No. of hours: 9

1. Aerobic respiration - ETS and oxidative phosphorylation
2. Anaerobic respiration, chemoautotrophy - oxidation of inorganic compounds - N, S, Fe and H.
3. Fermentative modes in microorganisms with special reference to alcoholic, Lactic acid fermentations

UNIT V: Bacterial Photosynthesis No. of hours:9

1. Photosynthetic pigments, Photosynthetic apparatus in prokaryotes
2. Outline of oxygenic photosynthesis in bacteria
3. Outline of anoxygenic photosynthesis in bacteria

IV SEMESTER
COURSE 10: - MICROBIAL PHYSIOLOGY AND METABOLISM

Credits -1

1. Effect of Temperature on bacterial growth 2.Effect of pH on bacterial growth
2. Colony count in Plates
3. Study and plot the growth curve of E. coli by turbidometric and standard plate count methods
4. Observation and identification of permanent slides of cyanobacteria

IV References:

1. Berg JM, Tymoczko JL and Stryer L (2011) Biochemistry, W.H.Freeman and Company Caldwell, D.R. (1995). Microbial Physiology and Metabolism, W.C. Brown Publications,Iowa, USA.
2. Lehninger, A.L., Nelson, D.L. and Cox, M.M. (1993). Principles of Biochemistry, 2 nd Edition, CBS Publishers and Distributors, New Delhi.
3. Sashidhara Rao, B. and Deshpande, V. (2007). Experimental Biochemistry: A student Companion. I.K. International Pvt. Ltd.
4. Tymoczko JL, Berg JM and Stryer L (2012) Biochemistry: A short course, 2nd ed., W.H.Freeman
5. Voet,D. and Voet J.G (2004) Biochemistry 3rd edition, John Wiley and Sons
6. White, D. (1995). The Physiology and Biochemistry of Prokaryotes, Oxford University Press, New York.

V Co-Curricular Activities:

1. Assignments in nutrient utilization, energy production, metabolic pathways,
2. Students can study microbial growth curves, metabolic pathways, or physiological responses to environmental factors.
3. Organize seminars where students can deliver presentations on specific topics in microbial physiology and metabolism.
4. Create visual representations of microbial metabolic pathways.

UNIT I: Microbial Nutrition

Nutritional Requirements of Microorganisms

Microorganisms require various nutrients to grow, reproduce, and perform metabolic functions. These nutrients are divided into **macronutrients** and **micronutrients**, with specific examples and functions for each.

Macronutrients

- **Macronutrients** are needed in large amounts for cell structure and metabolism.

1. **Carbon (C)**
    - Primary source of energy and building blocks for microbial cells.
    - **Source**: Organic compounds (heterotrophs) or carbon dioxide (autotrophs).
    - **Example**: *Escherichia coli* uses glucose (organic carbon) for growth.
2. **Nitrogen (N)**
    - Essential for amino acids, proteins, nucleic acids, and other cellular components.
    - **Source**: Ammonium ions (NH4+), nitrates (NO3), nitrogen gas (N2), or organic nitrogen sources.
    - **Example**: *Azotobacter* fixes nitrogen gas for its growth.
3. **Phosphorus (P)**
    - Vital for DNA, RNA, ATP, and phospholipids.
    - **Source**: Phosphate salts (PO4).
    - **Example**: *Bacillus subtilis* requires phosphate for cell wall synthesis.
4. **Sulfur (S)**
    - Important for amino acids like cysteine and methionine and coenzymes like CoA.
    - **Source**: Sulfate (SO4), hydrogen sulfide (H2S), organic sulfur compounds.
    - **Example**: *Desulfovibrio* uses sulfate as an electron acceptor in anaerobic respiration.
5. **Potassium (K), Magnesium (Mg), Calcium (Ca)**
    - Required for enzyme activity, stability of ribosomes, and membrane function.
    - **Source**: Salts of potassium, magnesium, and calcium.
    - **Example**: *Staphylococcus aureus* requires magnesium for enzyme activation.

Micronutrients

- **Micronutrients** are needed in smaller amounts but are essential for enzymatic functions and growth.

1. **Iron (Fe)**
    - Key component of cytochromes and electron carriers in respiration.
    - **Source**: Iron salts, ferric and ferrous forms.
    - **Example**: *Escherichia coli* requires iron for aerobic and anaerobic respiration.
2. **Manganese (Mn), Zinc (Zn), Copper (Cu)**
    - Act as cofactors in enzyme systems.
    - **Example**: *Pseudomonas aeruginosa* requires zinc for protein synthesis.
3. **Trace Elements (e.g., Mo, Co, Ni)**
    - Important for specific enzymes (e.g., molybdenum in nitrogenase for nitrogen fixation).
    - **Example**: *Rhizobium* uses molybdenum for nitrogen fixation in symbiosis with plants.

Growth Factors

- Some microorganisms cannot synthesize certain compounds and need them from the environment. These are known as **growth factors**.

1. **Vitamins**
    - Serve as coenzymes or precursors for coenzymes.
    - **Example**: *Lactobacillus* species require B vitamins for fermentation.
2. **Amino Acids**
    - Essential for protein synthesis.
    - **Example**: *Saccharomyces cerevisiae* requires specific amino acids for growth.

Energy Sources

Microorganisms also require energy, which they obtain from different sources:

- **Phototrophs**: Use light energy (e.g., *Cyanobacteria*).
- **Chemotrophs**: Use chemical compounds (e.g., *Escherichia coli*).
- **Heterotrophs**: Obtain carbon from organic compounds (e.g., *Saccharomyces cerevisiae*).
- **Autotrophs**: Use inorganic carbon sources like CO2 (e.g., *Nitrosomonas* for nitrification).

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Methods of uptake of nutrients by cells- Primary and secondary active transport, concept of uniport, symport and antiport Group translocation; Iron uptake

Cells require nutrients and ions to carry out various metabolic processes. To acquire these molecules from their surroundings, cells use different mechanisms of transport. Among these, **active transport** is crucial for moving substances **against their concentration gradient**, requiring energy input. Active transport is divided into **primary active transport** and **secondary active transport**.

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1. Primary Active Transport

Primary active transport directly uses energy, usually in the form of **ATP**, to transport molecules across the cell membrane **against their concentration gradient** (from lower to higher concentration).

Mechanism:

- Transport proteins, called **pumps**, utilize ATP hydrolysis to change their conformation and move molecules or ions.
- This process generates a concentration gradient or electrochemical gradient across the membrane.

Key Features:

- **Energy source**: Direct ATP hydrolysis.
- **Direction of movement**: Against the concentration gradient.
- Involves **specific pumps**.

Example:

- **Na^+/K^+ ATPase pump**:
  Pumps **3 Na^+ ions** out of the cell and **2 K^+ ions** into the cell, maintaining the membrane potential and ionic balance.
- **Calcium pumps (Ca^{2+} ATPase)**:
  Move calcium ions out of the cytoplasm into the endoplasmic reticulum or extracellular space.

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2. Secondary Active Transport

Secondary active transport indirectly uses energy by utilizing the **pre-existing electrochemical gradient** created by primary active transport. It does not directly use ATP but relies on the movement of one molecule (usually an ion like Na^+ or H^+) **down its concentration gradient** to drive the movement of another molecule **against its gradient**.

Mechanism:

- Secondary active transport involves **cotransporters** that couple the movement of two substances:
    - **Symport**: Both substances move in the **same direction** across the membrane.
    - **Antiport**: Substances move in **opposite directions**.

Key Features:

- **Energy source**: Electrochemical gradient (established by primary active transport).
- **Direction of movement**: One molecule moves down its gradient, driving the other against its gradient.
- Involves **cotransporters** (symporters and antiporters).

Types of Active Transport

A. Uniport

- Transport of a single type of molecule in one direction across the membrane.
- **Example**:
    - **Proton pump (H+pump)**: Used by *Saccharomyces cerevisiae* to expel protons and maintain pH balance.

B. Symport

- Two or more molecules are transported in the same direction across the membrane.
- **Example**:
    - **Sodium-glucose symporter**: In humans, it transports glucose and sodium ions into intestinal cells together.

C. Antiport

- Two molecules are transported in opposite directions across the membrane.
- **Example**:

- **Sodium-calcium exchanger (Na+Ca2+ antiporter)**: In cardiac cells, it pumps sodium into the cell and calcium out, which is crucial for muscle contraction.

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3. Group Translocation

It is a type of active transport where the transported molecule is chemically modified as it crosses the membrane.

Example:

- **Phosphotransferase system (PTS)**: Used by bacteria like *Escherichia coli* to transport sugars like glucose. The glucose is phosphorylated during transport, converting it to glucose-6-phosphate.

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4. Iron Uptake

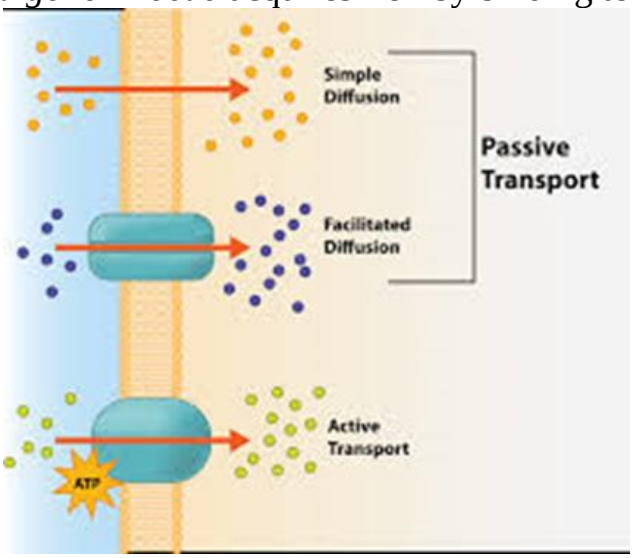
Microorganisms often need to acquire iron, which is essential for growth but usually scarce due to its binding with host proteins. Several methods are used to acquire iron.

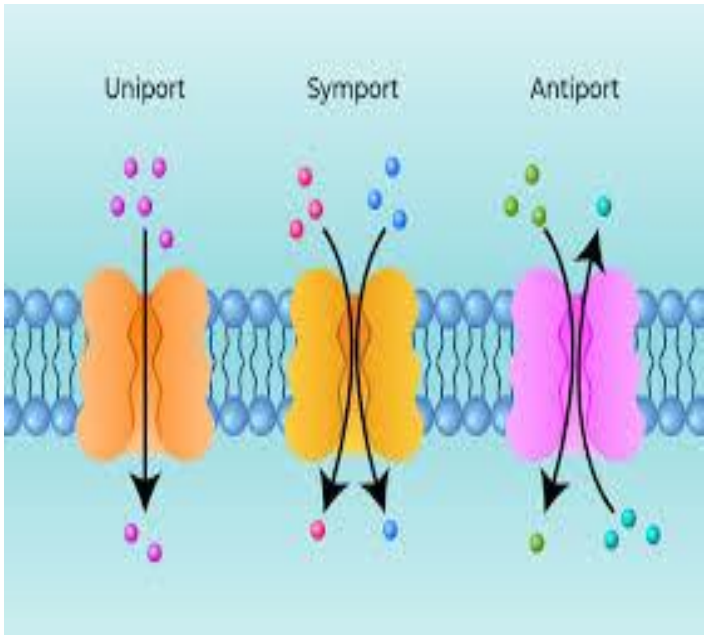
Siderophore-mediated uptake: Siderophores are molecules that bind iron tightly and transport it into the cell.

Example: *Pseudomonas aeruginosa* produces siderophores like pyoverdine to scavenge iron from the environment.

Direct uptake of iron-binding proteins: Some bacteria directly take up iron from host proteins like transferrin or lactoferrin.

Example: *Neisseria gonorrhoeae* acquires iron by binding to transferrin.





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Nutritional Groups of Microorganisms Based on Carbon, Energy, and Electron Sources

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1. Carbon Source
Microorganisms can be classified based on the source of carbon they use for growth.

A. Autotrophs

- **Carbon Source**: Use inorganic carbon, primarily in the form of carbon dioxide (CO_2).
- **Example**:
    1. **Cyanobacteria**: Use CO_2 and light energy for photosynthesis.
    2. **Nitrosomonas**: Use CO_2 as their carbon source for growth while oxidizing ammonia.

B. Heterotrophs

- **Carbon Source**: Use organic compounds (e.g., sugars, amino acids) as carbon sources.
- **Example**:
    1. **Escherichia coli**: Uses glucose (organic carbon) for growth.
    2. **Saccharomyces cerevisiae**: Uses organic compounds (e.g., sugars) for fermentation.

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2. Energy Source
Microorganisms are classified based on how they obtain energy.

A. Phototrophs
- **Energy Source**: Light energy.
- **Example**:
    1. **Cyanobacteria**: Use light energy to drive photosynthesis.
    2. **Purple sulfur bacteria**: Use light for energy, typically in anaerobic conditions.

B. Chemotrophs
- **Energy Source**: Chemical compounds (organic or inorganic) for energy.
- **Example**:
    1. **Escherichia coli**: Uses organic compounds like glucose in aerobic or anaerobic respiration.
    2. **Thermoproteus**: An extremophile that oxidizes sulfur for energy.

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3. Electron Source
Microorganisms are also classified based on the source of electrons they use in their metabolic processes.

A. Lithotrophs
- **Electron Source**: Inorganic compounds.
- **Example**:
    1. **Nitrosomonas**: Oxidizes ammonia to nitrite, using it as an electron donor.
    2. **Sulfolobus**: Oxidizes sulfur compounds for electrons.

B. Organotrophs
- **Electron Source**: Organic compounds.
- **Example**:
    1. **Saccharomyces cerevisiae**: Uses organic compounds (e.g., glucose) to provide electrons during fermentation.
    2. **Pseudomonas aeruginosa**: Uses organic compounds as electron donors during respiration.

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4. Summary of Nutritional Groups
- **Autotrophs**: Use CO_2 as carbon source (e.g., *Cyanobacteria*, *Nitrosomonas*).
- **Heterotrophs**: Use organic carbon compounds (e.g., *E. coli*, *Saccharomyces cerevisiae*).

- **Phototrophs**: Use light as an energy source (e.g., *Cyanobacteria*, *Purple sulfur bacteria*).
- **Chemotrophs**: Use chemical compounds as energy sources (e.g., *E. coli*, *Thermoproteus*).
- **Lithotrophs**: Use inorganic compounds as electron donors (e.g., *Nitrosomonas*, *Sulfolobus*).
- **Organotrophs**: Use organic compounds as electron donors (e.g., *Saccharomyces cerevisiae*, *Pseudomonas aeruginosa*).

These classifications help understand how microorganisms obtain their energy, carbon, and electrons, which is essential for studying their metabolism and ecological roles.

Table 6.2: Nutritional classes of Microorganisms

Nutritional class	Energy/Electron/Carbon source	Organisms
Photoautotrophs	Light energy Inorganic e^- donor CO_2	Cyanobacteria, Purple and Green sulphur Bacteria
Photoheterotrophs	Light energy Organic e^- donor Organic carbon source	Purple and Green Nonsulfur bacteria
Chemoautotrophs	Inorganic chemical compounds as energy source Inorganic e^- donor CO_2	Nitrifying bacteria, Iron bacteria
Chemoheterotrophs	Organic compounds as energy, electron and carbon source.	Most pathogenic bacteria, fungi and protozoa.

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Growth media - synthetic, nonsynthetic, selective, enrichment and differential media

Growth media are used to cultivate microorganisms in the laboratory. They provide the necessary nutrients and conditions for microbial growth. Based on composition, selective properties, and function, growth media can be classified into several types:

1. Synthetic (Defined) Media

Media that contains all known chemical components in precise amounts. All ingredients are of known chemical composition.

It is Used to study the specific nutritional requirements of microorganisms.

Examples:

1. **Minimal salt medium**: Contains only essential salts, carbon sources (e.g., glucose), and nitrogen for microbial growth.
2. **Glucose-salts medium**: Provides a single carbon source (glucose) and essential minerals and salts for microbial growth.

2. Nonsynthetic (Complex) Media

Media that contain one or more ingredients of unknown composition, often derived from natural sources (e.g., yeast extract, beef extract).

It is Used for general cultivation of microorganisms that do not have specific nutritional requirements.

Examples:

1. **Nutrient agar**: Contains peptones, beef extract, and agar, commonly used for cultivating a wide range of bacteria.
2. **Tryptic soy agar**: Contains casein peptones, soybean peptones, and agar, used for general bacterial growth.

3. Selective Media

Media that contain specific ingredients that inhibit the growth of certain microorganisms, allowing only a particular group to grow.

It Used to isolate a specific microorganism from a mixture by suppressing the growth of unwanted species.

Examples:

1. **MacConkey agar**: Contains bile salts and crystal violet to inhibit the growth of Gram-positive bacteria, allowing Gram-negative bacteria (especially enteric bacteria) to grow.

2. **Sabouraud agar**: Contains low pH and antibiotics to selectively grow fungi while inhibiting bacterial growth.

4. Enrichment Media

Media that favor the growth of a particular microorganism from a mixture by providing special nutrients or conditions. Unlike selective media, enrichment media do not inhibit other microbes but provide conditions that enhance the growth of the target microorganism.

It is Used for isolating microorganisms from samples containing many different types of bacteria.

Examples:

1. **Selenite broth**: Enriches for *Salmonella* species by allowing them to grow while inhibiting the growth of other bacteria.
2. **Blood agar**: Enriches for fastidious organisms (e.g., *Streptococcus* species) that require blood for growth.

5. Differential Media

Media that allow the differentiation of microorganisms based on their metabolic activities. These media contain indicators (e.g., pH indicators) that change color in response to microbial activity.

It Used to differentiate between closely related microorganisms based on certain biochemical reactions.

Examples:

1. **MacConkey agar**: Contains lactose and a pH indicator to differentiate between lactose fermenters (e.g., *E. coli*, which turn pink) and non-fermenters (e.g., *Salmonella*, which remain colorless).
2. **Eosin methylene blue (EMB) agar**: Differentiates based on lactose fermentation; *E. coli* forms metallic green colonies, while other organisms form colorless colonies.

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UNIT II: Microbial Growth

1. Microbial Growth

Microbial growth refers to the **increase in the number of cells** in a microbial population rather than an increase in the size of individual cells. It typically occurs through **binary fission** in bacteria, where one cell divides into two identical daughter cells.

- Growth can be measured in terms of **cell number** or **biomass** over time.
- Microbial growth is often represented as a **growth curve** with four phases: lag, exponential (log), stationary, and death phase.

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2. Generation Time

Generation time is the **time required for a microbial population to double in number** under specific growth conditions.

- It is also known as **doubling time**.
- Generation time varies depending on the microbial species and environmental conditions like temperature, pH, and nutrient availability.

Formula:

$$\text{Generation time (G)} = \frac{\text{time}}{\text{number of generations}}$$

Example: For *Escherichia coli* in ideal conditions, the generation time is approximately **20 minutes**.

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3. Specific Growth Rate (μ)

Definition:
The specific growth rate is the **rate at which a microbial population grows** per unit of biomass over time, expressed as a **fraction of the population size**.

- It represents how quickly cells are dividing and increasing in number.
- It is typically used in the **exponential (log) phase** of growth.

Formula:

$$\mu = \frac{1}{X}\frac{dX}{dt}$$

Where:

- μ = specific growth rate (h^{-1})
- X = biomass concentration or population size
- dx/dt = rate of change in biomass concentration

Summary Table

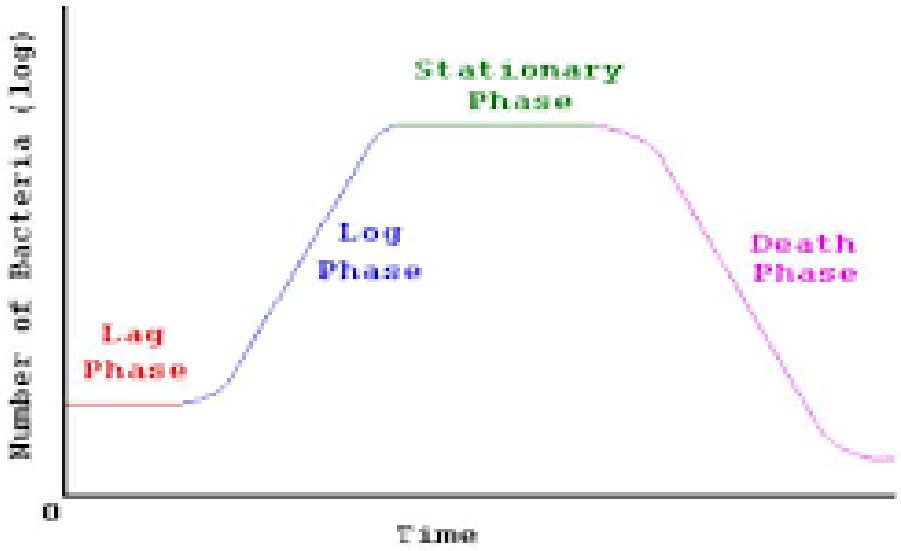
Term	Definition	Key Point
Microbial Growth	Increase in the number of microbial cells in a population.	Measured as cell number or biomass.
Generation Time	Time taken for a microbial population to double in number.	Depends on species and environment.
Specific Growth Rate (μ)	Rate of microbial population growth per unit of biomass over time.	Describes growth during the exponential phase.

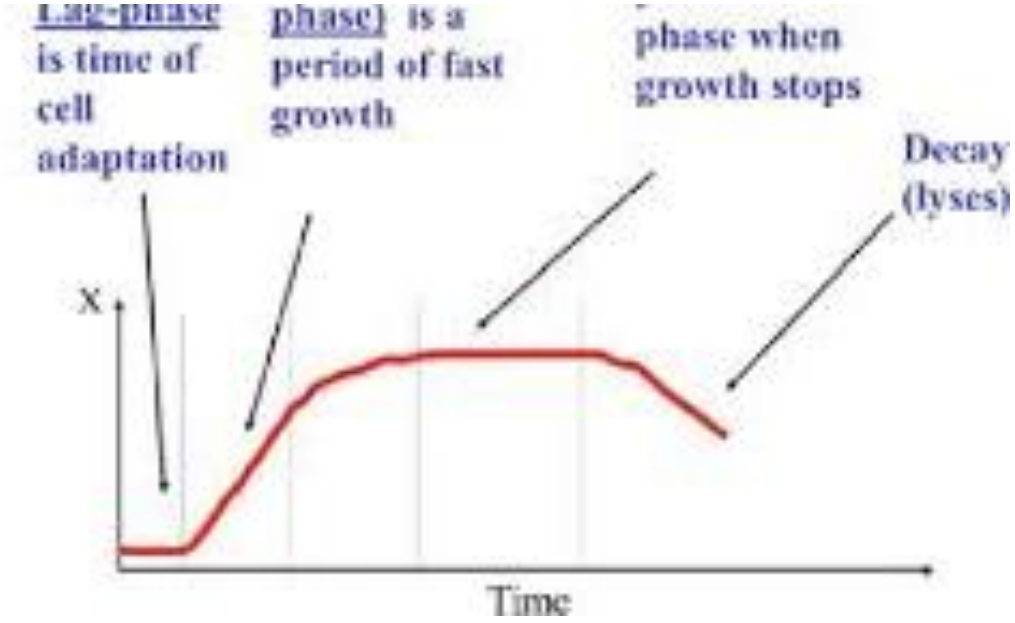
2. Phases of Growth in Batch Culture

Batch culture is a closed system where microbes grow in a fixed volume of nutrient medium. The growth curve has four distinct phases:

1. **Lag Phase:**
    - Cells adapt to new conditions, synthesize enzymes, and prepare for division.
    - No increase in cell number.
    - Example: *Bacillus subtilis* synthesizing amylase in a starch medium.
2. **Exponential (Log) Phase:**
    - Cells divide at their maximum rate, with constant generation time.
    - Growth is balanced and proportional to nutrient availability.
    - Example: Rapid growth of *E. coli* in a nutrient-rich medium.
3. **Stationary Phase:**
    - Growth rate slows due to nutrient depletion or accumulation of toxic products.
    - Population reaches a plateau where cell division equals cell death.
    - Example: Yeast cells accumulating ethanol in fermentation.
4. **Death Phase:**

- Cells die at an exponential rate due to prolonged stress or lack of nutrients.
- Example: *Pseudomonas aeruginosa* in a medium with no oxygen.





Applications of Growth Phases:

- Understanding phases helps optimize fermentation, antibiotic production, and bioreactor operations.

Synchronous, continuous, biphasic growth

1. Synchronous Growth

Synchronous growth occurs when all bacterial cells in a population divide **simultaneously** and are at the **same stage** of the cell cycle. This results in synchronized cycles of growth and division.

Key Characteristics:

- Cells divide at the same time, leading to **stepwise increases** in cell number.
- Achieved **experimentally** through environmental manipulation (e.g., nutrient starvation, temperature shifts).

Example:
When bacterial cells are subjected to a periodic supply of fresh nutrients or temperature shifts, they can be synchronized artificially. Such experiments are used to study bacterial cell division and gene expression.

2. Continuous Growth

Continuous growth occurs when bacterial cells are grown in a system where **fresh nutrients are continuously supplied**, and **waste products are removed**, maintaining a **constant growth rate** and steady state.

Key Characteristics:

- The bacteria remain in the **exponential phase** of growth.
- Achieved using a **chemostat**, a device that controls nutrient flow and waste removal.
- Useful for industrial processes and studying microbial physiology under steady conditions.

Example:
In a **chemostat culture**, *E. coli* can be grown continuously to produce **enzymes,**

antibiotics, or biofuels. Industrial fermentation processes use this method to maximize product yield.

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3. Biphasic Growth

Biphasic growth occurs when bacteria grow in the presence of **two carbon sources**. The growth curve displays **two different exponential phases**, separated by a **lag phase**. Initially, bacteria metabolize the **preferred nutrient**, and after its depletion, they switch to the secondary nutrient.

Key Characteristics:

- Two growth phases:
    1. Rapid growth using the preferred nutrient.
    2. Lag phase (enzyme synthesis).
    3. Slower growth using the secondary nutrient.
- Demonstrates bacterial adaptation to multiple energy sources.

Example:
When *E. coli* is grown in a medium containing both **glucose** and **lactose**:

- **Example:**
    - *E. coli* in a medium with glucose and lactose:
        - First phase: Glucose is consumed (exponential growth).
        - Lag phase: Enzymes (β-galactosidase) for lactose metabolism are produced.
        - Second phase: Lactose is consumed

Summary Table

Growth Type	Definition	Key Features	Example
Synchronous Growth	All cells divide simultaneously.	Stepwise increases in cell number.	Synchronized cultures in controlled labs.
Continuous Growth	Continuous nutrient supply, waste removal.	Steady-state exponential growth.	Chemostat culture for enzyme production.
Biphasic Growth	Growth in presence of two carbon sources.	Two exponential phases with a lag in between.	*E. coli* on glucose and lactose medium.

Applications:

- Synchronous growth aids cell cycle studies.
- Continuous growth is essential in industrial fermentation.
- Biphasic growth provides insights into metabolic regulation and enzyme induction.

Factors influencing microbial growth

Microbial growth is influenced by several environmental, chemical, and physical factors. These factors affect how microorganisms grow, divide, and function.

Physical Factors **directly affect microbial growth.**

Temperature:
Microorganisms have an **optimal temperature range** for growth:

- **Psychrophiles**: Grow at low temperatures (0–20°C). Example: *Pseudomonas*.
- **Mesophiles**: Grow at moderate temperatures (20–45°C). Example: *E. coli*.
- **Thermophiles**: Grow at high temperatures (45–80°C). Example: *Bacillus stearothermophilus*.
- **Hyperthermophiles**: Grow at extreme temperatures (>80°C). Example: *Thermococcus*.

pH:
Microbial growth depends on the hydrogen ion concentration:

- **Acidophiles**: Grow in acidic environments (pH < 6). Example: *Lactobacillus*.
- **Neutrophiles**: Grow in neutral pH (pH 6–8). Example: *E. coli*.
- **Alkaliphiles**: Grow in basic environments (pH > 8). Example: *Bacillus alcalophilus*.

Oxygen Requirement:
Microorganisms can be classified based on their oxygen needs:

- **Obligate aerobes**: Require oxygen for growth. Example: *Mycobacterium tuberculosis*.
- **Facultative anaerobes**: Grow with or without oxygen. Example: *E. coli*.
- **Obligate anaerobes**: Cannot survive in oxygen. Example: *Clostridium botulinum*.
- **Aerotolerant anaerobes**: Tolerate but do not use oxygen. Example: *Lactobacillus*.

- **Microaerophiles**: Require low oxygen levels. Example: *Helicobacter pylori*.

Light:
Light influences **photosynthetic microbes** like cyanobacteria and algae that require light for energy production.

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2. Chemical Factors

1. **Nutrient Availability:**
   - Essential nutrients (C, N, P, S, etc.) influence growth.
   - Examples:
     - *Cyanobacteria* need light and CO2 for photosynthesis.
     - *Pseudomonas fluorescens* grows in environments rich in organic carbon.
2. **Salinity (Osmotic Pressure):**
   - Microbes vary in salt tolerance: halophiles thrive in high salinity.
   - Examples:
     - *Halobacterium salinarum* grows in salt concentrations >20%.
     - *E. coli* cannot tolerate high salt concentrations.

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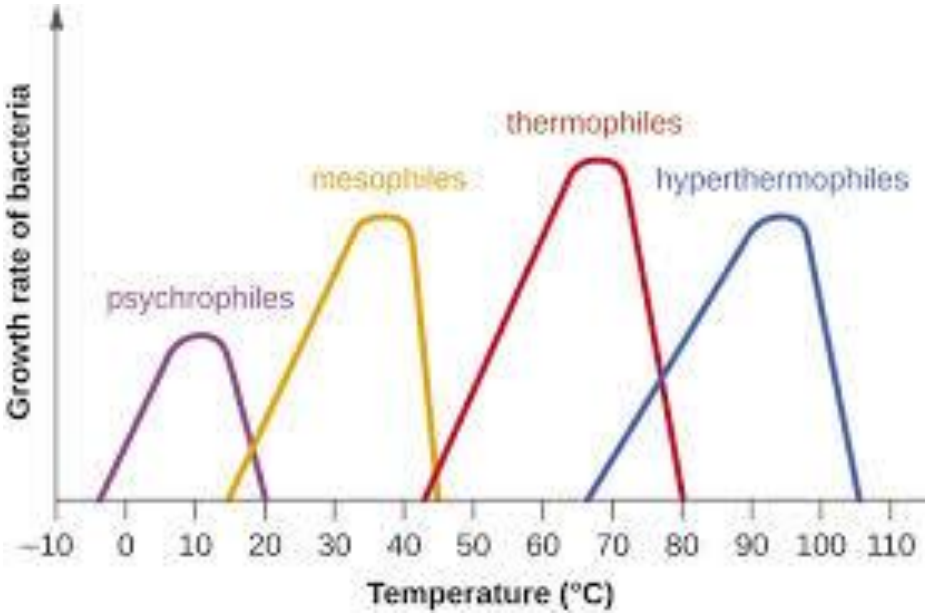
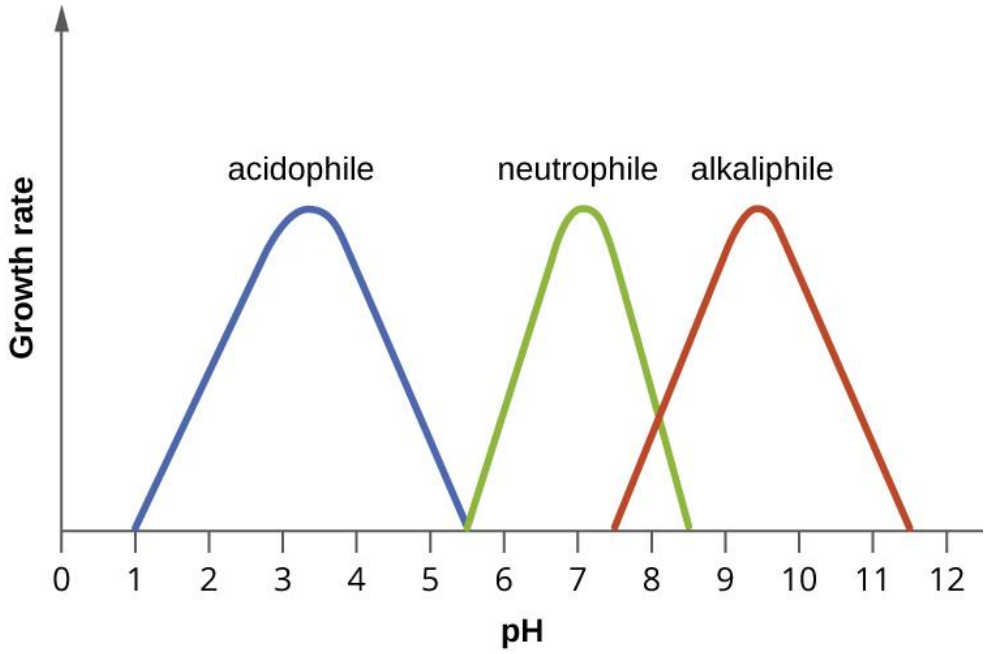
3. Biological Factors

1. **Competition:**
   - Interactions with other microbes can affect growth.
   - Examples:
     - *Streptomyces* produces antibiotics to inhibit competitors.
     - *Lactobacillus* inhibits pathogens in the gut.
2. **Symbiosis:**
   - Microbes grow in association with hosts or other organisms.
   - Examples:
     - Rhizobia fix nitrogen in legume roots.
     - *Mycorrhiza* enhances plant nutrient uptake.


Growth rate of bacteria
psychrophiles
mesophiles
thermophiles
hyperthermophiles
–10 0 10 20 30 40 50 60 70 80 90 100 110
Temperature (°C)


Growth rate
acidophile
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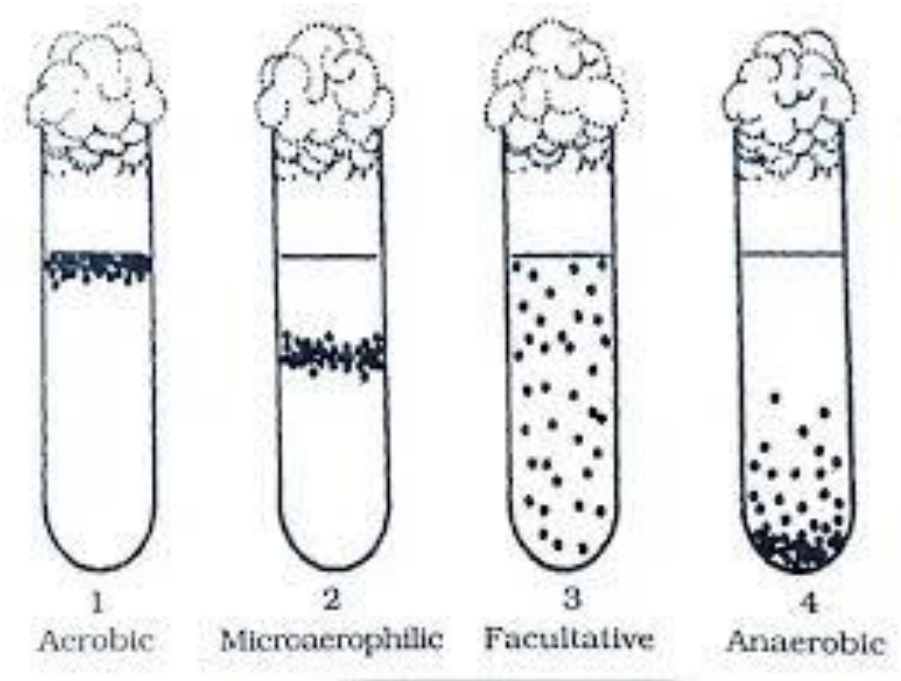
0 1 2 3 4 5 6 7 8 9 10 11 12
pH



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Methods for Measuring Microbial Growth

Measuring microbial growth is essential for understanding the growth of microorganisms in both laboratory and industrial settings. There are various methods to measure microbial growth.

- Direct microscopy
- Viable count estimates
- Turbidometry
- Biomass measurement

1. Direct Microscopy (Total Cell Count)

Direct microscopy involves counting the microorganisms directly using a **microscope**. This method does not differentiate between living and dead cells, so it provides a total count of all cells in the sample.

Procedure:

- A small sample of microbial culture is placed on a **microscope slide**.
- The sample is stained with a dye (optional) to improve visibility.

- Cells are counted manually or using an automated counting system under a microscope, often using a **hemocytometer** or **Petroff-Hausser counting chamber**.

Advantages:

- Provides an immediate count of all microorganisms in a sample.
- Simple and inexpensive.

Disadvantages:

- Does not differentiate between living and dead cells.
- Not suitable for highly turbid or dense cultures.

Example:

- *Escherichia coli* culture can be counted by direct microscopy to assess the total number of cells in the culture.

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2. Viable Count Estimates (Plate Count Method)

The viable count method involves determining the number of **living** cells in a sample by plating a diluted portion onto an agar plate and counting the **colony-forming units (CFUs)** that grow.

Procedure:

- A sample is serially diluted in a sterile buffer or saline solution.
- A small volume of each dilution is plated onto agar plates.
- The plates are incubated to allow growth.
- After incubation, colonies are counted, and the number of viable microorganisms is determined by multiplying the number of colonies by the dilution factor.

Advantages:

- Provides an estimate of the number of **viable, metabolically active** cells.
- Reliable for both bacterial and fungal cultures.

Disadvantages:

- Requires incubation time, which can take hours or days depending on the organism.

- Some cells may be viable but non-culturable (VNC) and won't form colonies.

Example:

- *Streptococcus pneumoniae* cultures can be counted by plating dilutions onto agar and counting the number of colonies that form.

---

3. Turbidometry (Optical Density)

Turbidometry measures the **cloudiness** or **turbidity** of a microbial culture, which correlates with the number of cells present. The more cells in the sample, the greater the turbidity.

Procedure:

- A microbial culture is placed in a test tube or cuvette.
- The turbidity is measured using a **spectrophotometer** at a specific wavelength (usually 600 nm).
- The **optical density (OD)** is recorded, which correlates with the cell concentration in the culture.

Advantages:

- Fast and easy to perform.
- Provides continuous, real-time measurements of growth.

Disadvantages:

- Measures **total biomass**, not just viable cells, so dead cells also contribute to turbidity.
- Accuracy can be affected by the size or shape of the cells.

Example:

- *Bacillus subtilis* cultures can be monitored for growth by measuring turbidity at 600 nm, where higher OD values indicate more bacterial growth.

---

4. Biomass Measurement (Dry Weight or Wet Weight)

Biomass measurement involves quantifying the **total mass** of microbial cells in a culture, typically through **dry weight** or **wet weight** measurements.

Procedure:

- **Wet weight**: The biomass is collected by centrifuging the culture, then weighing the cell pellet directly.
- **Dry weight**: After collecting the biomass, the cells are dried in an oven to remove water, and the dry mass is weighed.
- The weight of the microbial biomass can be used to estimate the growth of the population.

Advantages:

- Provides a direct measure of **total microbial mass**.
- Useful for measuring growth in systems where cell numbers do not correlate well with other methods (e.g., filamentous fungi).

Disadvantages:

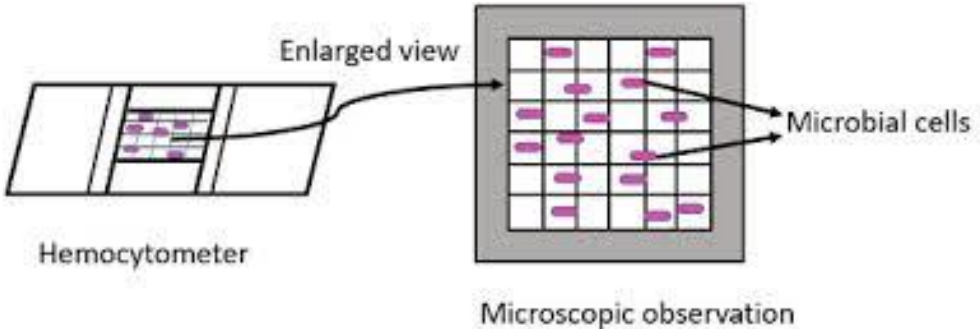
- Time-consuming (especially the dry weight method).
- Not as widely used as other methods for routine microbial growth measurement.
- Only measures **biomass**, not viable cells.

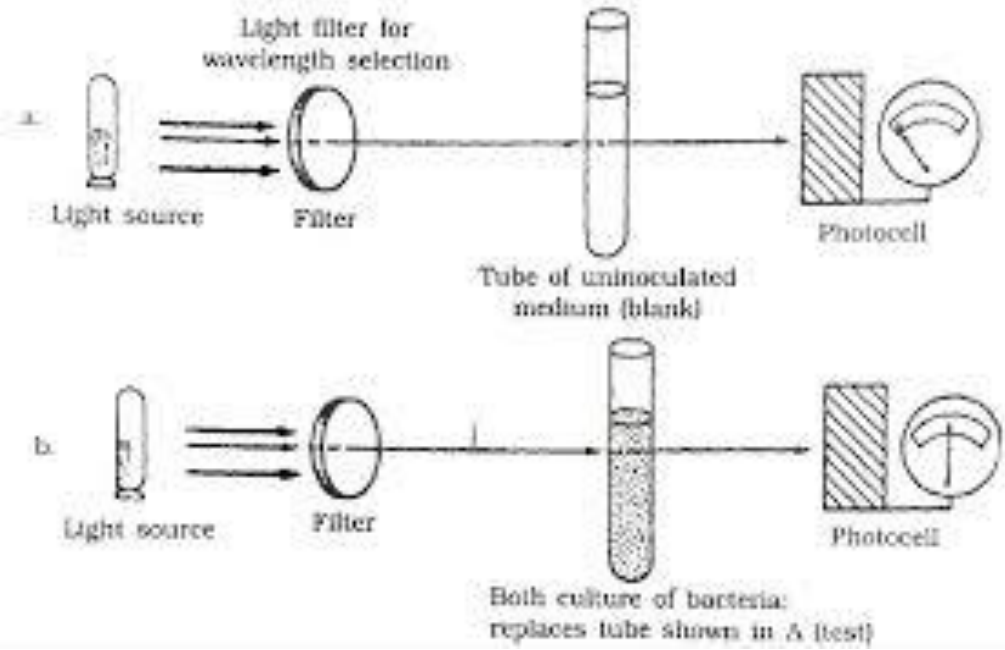
Example:

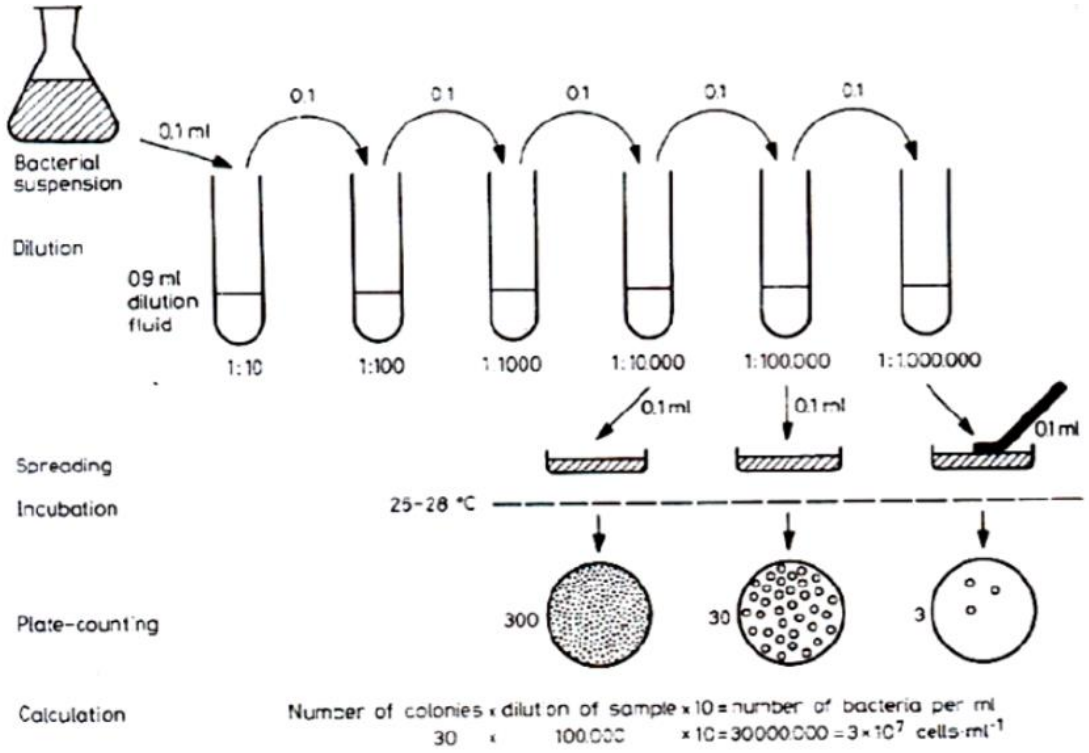
- *Aspergillus niger*, a fungal species, can be measured for growth by assessing the dry weight of the fungal mass after a culture period.

Applications:

- Direct microscopy is used in research and diagnostic labs for quick estimates.
- Viable count is critical in clinical microbiology and food industry.
- Turbidometry is often used in industrial fermentation for continuous growth monitoring.
- Biomass measurement is important in bioprocesses for assessing production yield.


Enlarged view
Microbial cells
Hemocytometer
Microscopic observation


Light filter for wavelength selection
Light source
Filter
Photocell
Tube of uninoculated medium (blank)
b.
Light source
Filter
Photocell
Both culture of bacteria: replaces tube shown in A (test)


Bacterial suspension
0.1 ml
0.1
0.1
0.1
0.1
0.1
Dilution
0.9 ml dilution fluid
1:10
1:100
1:1000
1:10.000
1:100.000
1:1.000.000
0.1 ml
0.1 ml
0.1 ml
Spreading
Incubation
25-28 °C
Plate-counting
300
30
3
Calculation
Number of colonies x dilution of sample x 10 = number of bacteria per ml
30 x 100.000 x 10 = 30000.000 = 3 x 10^7 cells·ml^-1

UNIT III: THERMODYNAMICS, BREAKDOWN OF CARBOHYDRATES

1. Free Energy (Gibbs Free Energy, G)

Free energy is the amount of energy in a system that is **available to do work** under constant temperature and pressure. It determines whether a reaction is **spontaneous** or not.

Gibbs Free Energy (G) is the energy available to do work in a biological system.

Equation: $\Delta G = \Delta H - T\Delta S$

- Where:
  ΔG: Change in free energy
  ΔH: Change in enthalpy
  T: Temperature (Kelvin)
  ΔS: Change in entropy

The change in free energy (ΔG\Delta GΔG) is used to predict the direction of a chemical reaction:

- If $\Delta G < 0$: Reaction is **spontaneous** (exergonic). Example: ATP hydrolysis.
- If $\Delta G > 0$: Reaction is **non-spontaneous** (endergonic). Example: Synthesis of glucose in photosynthesis.

---

2. Enthalpy (H)

Enthalpy is the total **heat content** of a system. It represents the energy required to **break bonds** and the energy released when new bonds are formed during a chemical reaction.

- The change in enthalpy (ΔH\Delta HΔH) indicates whether a reaction absorbs or releases heat:
  - $\Delta H < 0$: Exothermic reaction (heat is released).
  - $\Delta H > 0$: Endothermic reaction (heat is absorbed).

Example:

- **Combustion of methane** (CH_4) is an exothermic reaction:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (\Delta H = -890\ kJ/mol)$$

The negative ΔH indicates that heat is released during the reaction.

---

3. Standard Free Energy Change (ΔG)
The standard free energy change (ΔG) is the change in Gibbs free energy for a reaction under **standard conditions**:

- Temperature = 25 C 298K
- Pressure = 1 atm1
- Reactants and products at 1 M concentration.

Equation:

ΔG∘=−RTln⁡Keq\Delta G^\circ = -RT \ln K_{\text{eq}}ΔG∘=−RTlnKeq
Where:

- R: Universal gas constant (8.314 J/mol)
- T: Temperature in Kelvin
- Keq: Equilibrium constant

Example:

- **Glucose oxidation**:

C6H12O6+6O2→6CO2+6H2O (ΔG∘=−2870 kJ/mol)
The negative ΔG∘\Delta G^\circΔG∘ indicates that the reaction is **highly spontaneous** under standard conditions.

---

4. Entropy (S)
Entropy is a measure of the **disorder** or **randomness** in a system. Higher entropy means greater disorder.

- The change in entropy (ΔS\Delta SΔS) indicates whether a system becomes more or less disordered:
  - ΔS>0: Increase in disorder (favorable).
  - ΔS<0: Decrease in disorder (unfavorable).

Example:

- **Melting of ice**:

H2O(solid)→H2O(liquid)
When ice melts, the structure of water molecules becomes more disordered, so ΔS>0.

- **Dissolution of NaCl in water**:

NaCl(solid)→Na(aq)+

When salt dissolves, the ions disperse into solution, increasing disorder, so $\Delta S>0$.

Conclusion

The **free energy**, **enthalpy**, **standard free energy change**, and **entropy** are critical thermodynamic concepts that govern chemical and biological processes. Understanding these parameters allows us to predict the feasibility and spontaneity of reactions, which is essential in microbiology, biochemistry, and energy-related studies.

Examples in Biological Systems

- **ATP Hydrolysis**: Drives energy-requiring processes like muscle contraction ($\Delta G<0$).
- **Protein Folding**: Driven by a balance of enthalpy and entropy; unfolded states are disordered (S), but folded structures are stabilized by interactions (H).
- **Photosynthesis**: Light energy reduces carbon dioxide into glucose ($\Delta G>0$, requiring energy input).

These principles underpin metabolism, enzymatic activity, and ot her biochemical processes.

****

Explain First and Second law of Thermodynamics. Open and Closed system.

First Law of Thermodynamics (Law of Energy Conservation)

The first law states that **energy cannot be created or destroyed**, only converted from one form to another. The total amount of energy in a system remains the same.

Example:

- When you eat food, the chemical energy in the food is converted into energy for movement, body heat, and other activities.
- In a car engine, chemical energy from fuel is converted into mechanical energy to move the car.

Applications:

- **Power plants:** Convert chemical energy (fuel) into electrical energy.
- **Human body:** Converts food energy into kinetic and heat energy.
- **Batteries:** Store chemical energy and convert it into electrical energy when used.

The **first law of thermodynamics**:

$$\Delta U = Q - W$$

Where:

- ΔU = Change in internal energy of the system
- Q = Heat added to the system
- W = Work done by the system

This equation expresses the principle of **energy conservation**: the energy added as heat is partly used to increase internal energy and partly to do work.

Second Law of Thermodynamics (Law of Entropy)

The second law states that **energy naturally flows from a high-energy state to a low-energy state**, increasing disorder or **entropy** in the system. In simple words, energy conversions are not 100% efficient—some energy is always lost as heat.

The **second law of thermodynamics** :

$$\Delta S \geq \frac{Q}{T}$$

Where:

- ΔS = Change in entropy of the system
- Q = Heat added to the system (reversibly)
- T = Absolute temperature (in Kelvin) at which the heat is added

For a **reversible process**, the equation becomes:

$$\Delta S = \frac{Q}{T}$$

For an **irreversible process**, the inequality applies:

$$\Delta S > \frac{Q}{T}$$

This law states that the **entropy of an isolated system always increases** in an irreversible process and remains constant in a reversible process, reflecting the **direction of natural processes** and the **inevitable increase in disorder**.

Example:

- Ice melting in a warm room: Heat energy flows from the room to the ice, causing it to melt.
- A car engine releases heat energy into the air while running.

Applications:

- **Refrigerators:** Work by removing heat from the inside and releasing it outside.
- **Engines:** Inefficiency of fuel combustion results in heat loss, following the second law.
- **Ecosystems:** Energy transfers between organisms (like from plants to animals) lose some energy as heat.

Open and Closed Systems

Open System
An **open system** is a system where **both energy and matter** can enter or leave.

Examples:

- **Human body:** We eat food (matter) and release heat and energy.
- **Boiling pot of water without a lid:** Heat (energy) and steam (matter) escape into the air.

Applications:

- **Industrial plants:** Input raw materials and energy, output products and waste.
- **Living organisms:** Exchange gases, nutrients, and energy with their surroundings.
- **Climate systems:** Earth receives energy from the sun and radiates some back into space.

Closed System

A **closed system** allows **only energy** to enter or leave, **but not matter**.

Examples:

- **Earth:** Receives energy from the sun but hardly any matter from space.
- **Sealed bottle of soda:** Energy (like heat) can pass through the bottle, but the soda and gas inside stay contained.
- **Thermos flask:** Designed to prevent energy (heat) loss while keeping the contents (matter) inside.

Applications:

- **Greenhouses**
- **Pressure cookers**

Conclusion:
The **First Law of Thermodynamics** explains how energy is conserved, while the **Second Law** shows why energy transformations are not perfectly efficient. Understanding **open and closed systems** helps explain how energy and matter

flow in natural and artificial environments, playing essential roles in engineering, biology, and everyday life.

****

ATP and High-Energy Compounds

1. Structure of ATP (Adenosine Triphosphate)

- **Composition**:
  - **Adenine**: A nitrogenous base.
  - **Ribose**: A five-carbon sugar.
  - **Three Phosphate Groups**: Linked by high-energy phosphoanhydride bonds.
- **Key Features**:
  - The phosphate bonds are unstable and release energy upon hydrolysis.
  - The terminal phosphate bond is most commonly involved in energy transfer.

2. Properties of ATP

- **Energy Currency**: ATP stores and transfers energy in cells.
- **Hydrolysis Reaction**: $ATP+H2O \rightarrow ADP+Pi$
  - Releases energy to drive endergonic reactions.
- **Reversibility**: Can be regenerated from ADP and inorganic phosphate (Pi) via phosphorylation.

3. Standard Free Energy Change of ATP Hydrolysis

- **Biochemical Conditions**:
  - Actual free energy change (ΔG) in cells is more negative (-50 kJ/mol) due to higher ATP/ADP ratios.
- **Biological Relevance**:
  - Powers active transport (e.g., Na+-K+ pump), muscle contraction, and biosynthesis.

4. Other High-Energy Compounds

- **Phosphoenolpyruvate (PEP)**:
  - Example: Converts ADP to ATP during glycolysis.
- **Creatine Phosphate**:
  - Example: Serves as a quick energy reserve in muscle cells.
- **1,3-Bisphosphoglycerate (1,3-BPG)**:
  - Example: Participates in ATP generation in glycolysis.

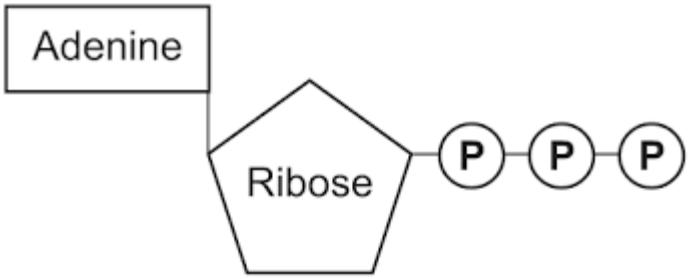
---

5. Examples of ATP-Driven Processes

- **Active Transport**:

    - ATP powers ion pumps, such as the sodium-potassium pump.
- **Mechanical Work**:
    - ATP hydrolysis fuels muscle contractions.
- **Chemical Work**:
    - ATP provides energy for synthesizing macromolecules (e.g., proteins, DNA).
- **Signal Transduction**:
    - ATP is a precursor for cyclic AMP (cAMP), a secondary messenger in cell signaling.

Summary
ATP is the central molecule for energy transfer in biological systems. Its hydrolysis provides the energy necessary for various cellular processes. Other high-energy compounds, such as PEP and creatine phosphate, also play critical roles in energy management.



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Biological oxidation-reduction reactions. Structure and Function of NAD and FAD

- **Oxidation**: Loss of electrons (or hydrogen).
- **Reduction**: Gain of electrons (or hydrogen).
- **Coupling**: Oxidation and reduction always occur together in redox reactions.
- **Biological Relevance**:
    - Redox reactions are crucial for energy production, as seen in cellular respiration and photosynthesis.
    - Electrons transferred during these reactions are often carried by cofactors like NAD+ and FAD.

Structure and Function of NAD and FAD

Nicotinamide Adenine Dinucleotide (NAD^+)

NAD^+ is a coenzyme found in all living cells that functions as an electron carrier in redox reactions. It is involved in energy metabolism by accepting and donating electrons in pathways such as glycolysis, the Krebs cycle, and oxidative phosphorylation.

Structure:

- NAD^+ is a coenzyme composed of two nucleotides:
  - One containing an adenine base.
  - The other containing nicotinamide (a derivative of vitamin B3 or niacin).
- The two nucleotides are joined by phosphate groups.

Function:

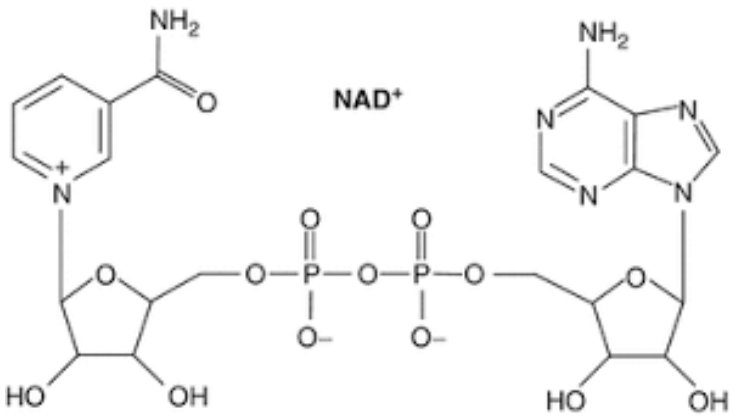
- NAD act as electron carriers in the Electron Transport Chain (ETC).
- Involved in cellular respiration, including **glycolysis, the Krebs cycle, and oxidative phosphorylation**.
- It Accepts electrons and becomes **NADH**, which then donates electrons to the electron transport chain (ETC) for ATP production.

Example:

- In glycolysis, **glyceraldehyde-3-phosphate dehydrogenase** transfers electrons to NAD^+, forming **NADH**, which later donates electrons in oxidative phosphorylation.

Biological Significance:

- Important for ATP production and energy metabolism.
- Helps in metabolic pathways like fermentation, fatty acid oxidation, and amino acid metabolism.
- NAD^+ is also involved in DNA repair .



Flavin Adenine Dinucleotide (FAD)

FAD is a redox-active coenzyme derived from riboflavin (vitamin B2) that plays a crucial role in cellular respiration. It functions as an electron carrier in metabolic reactions, particularly in the Krebs cycle and electron transport chain, where it exists in oxidized (FAD) and reduced ($FADH_2$) forms.

Structure:

- FAD consists of:
  - A riboflavin (vitamin B2) component.
  - An adenine nucleotide.
- It exists in two forms:
  - **Oxidized (FAD)** – Can accept electrons.
  - **Reduced ($FADH_2$)** – Carries high-energy electrons.

Function:

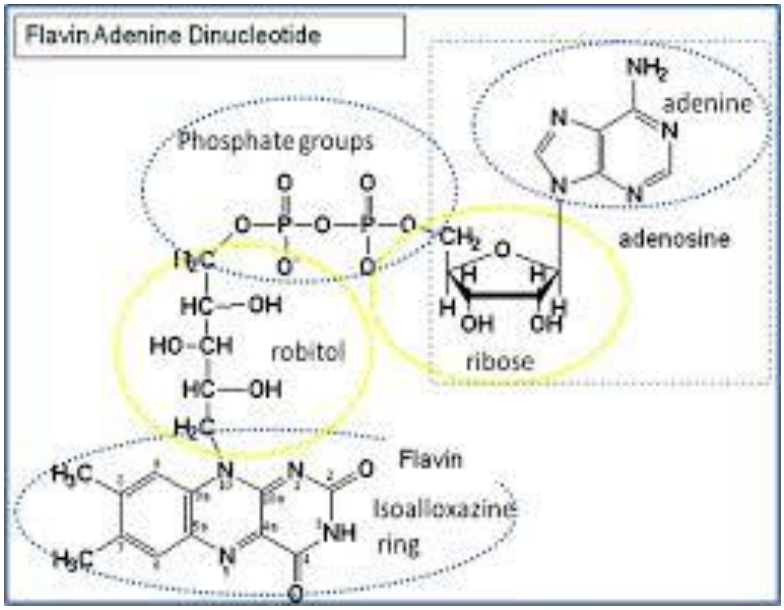
- FAD act as electron carriers in Krebs cycle & Electron Transport Chain (ETC).
- Participates in oxidative phosphorylation by donating electrons to the ETC, generating ATP.

Example:

- In the Krebs cycle, **succinate dehydrogenase** converts succinate to fumarate by transferring electrons to FAD, forming **$FADH_2$**, which later contributes to ATP generation.

Biological Significance:

- Important for cellular respiration and ATP synthesis.
- Involved in **β-oxidation of fatty acids, amino acid metabolism, and redox reactions**.
- Helps in detoxification reactions in peroxisomes.



Comparison of NAD and FAD

Feature	$NAD^+/NADH$	$FAD/FADH_2$
Derived from	Vitamin B3 (Niacin)	Vitamin B2 (Riboflavin)
Electron Transfer	Accepts 2 electrons, 1 proton	Accepts 2 electrons, 2 protons
Reduced Form	NADH	$FADH_2$
Role in Metabolism	Involved in glycolysis, Krebs cycle, oxidative phosphorylation	Involved in Krebs cycle, fatty acid oxidation
ATP Yield (ETC)	2.5 ATP per NADH	1.5 ATP per $FADH_2$

Conclusion
NAD and FAD are vital electron carriers in metabolism, helping generate ATP through **cellular respiration**.

****

GLYCOLYSIS

Glycolysis is an important metabolic pathway that breaks down glucose into pyruvate, generating ATP and NADH. It occurs in the cytoplasm of all living cells and plays a key role in energy production.

1. Glycolysis (EMP Pathway)

- Glycolysis is the **first step** in the breakdown of glucose, occurring in the cytoplasm of the cell, where one molecule of glucose ($C_6H_{12}O_6$) is converted into two molecules of pyruvate.
- **Pathway Name**: **Embden-Meyerhof-Parnas (EMP) pathway**.

Steps of Glycolysis (overview):

1. **Glucose phosphorylation**: Glucose is converted to **glucose-6-phosphate** (G6P) using 1 ATP.
2. **Isomerization and second phosphorylation**: G6P is converted to **fructose-6-phosphate**, then **fructose-1,6-bisphosphate** using another ATP.
3. **Cleavage**: Fructose-1,6-bisphosphate is split into **two three-carbon molecules**: dihydroxyacetone phosphate (DHAP) and **glyceraldehyde-3-phosphate (G3P)**.
4. **Energy generation**: G3P is oxidized, and the energy released is used to produce **ATP** and **NADH**.
5. **Final Products**: Two molecules of **pyruvate** are produced per molecule of glucose, along with 2 **ATP** (net) and 2 **NADH**.

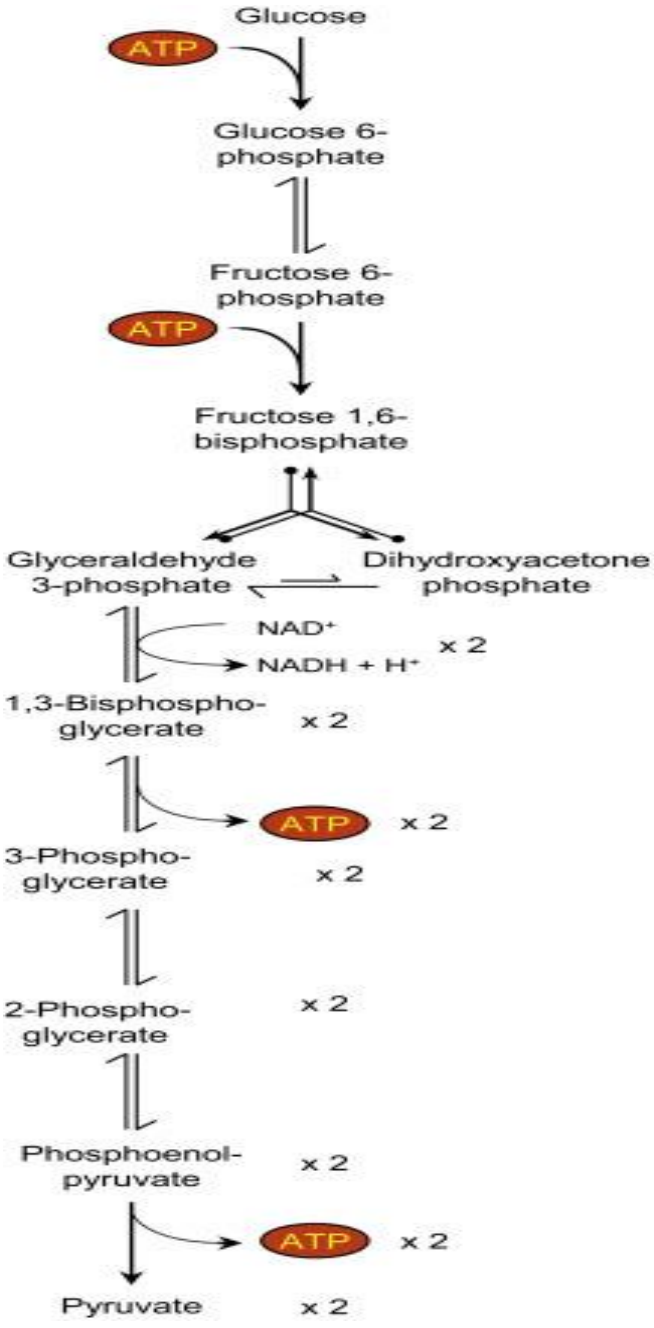
Significance:

- **Primary ATP Source:** Glycolysis Provides quick energy, especially in cells without mitochondria (e.g., RBCs).
- **Occurs in in Aerobic & Anaerobic Conditions:** Leads to the **Krebs cycle** in oxygen presence; forms **lactic acid (animals)** or **ethanol (yeast)** anaerobically.
- **Universal Pathway:** It is found in **bacteria, fungi, plants, and animals** .
- **Biosynthetic Precursors:** Supplies intermediates for **nucleotide, lipid, and amino acid synthesis**.
- **Supports Brain & Muscle Function:** Ensures **continuous ATP supply** for the brain and enables **rapid energy production** in muscles during intense activity.
- **Cancer Metabolism (Warburg Effect):** Cancer cells use glycolysis at a **high rate even with oxygen**, aiding rapid growth.

- **Industrial Applications:** Glycolysis is the basis of **alcohol fermentation (brewing industry)** and **lactic acid fermentation (dairy industry, yogurt production)**.

Conclusion:

Glycolysis is essential for **energy metabolism, biosynthesis, survival in anaerobic conditions**, and has medical and industrial significance.



Hexose Monophosphate Pathway (HMP Shunt / Pentose Phosphate Pathway)

- The **Pentose Phosphate Pathway (PPP)** or **HMP shunt** is a metabolic pathway parallel to glycolysis. It serves primarily to produce **NADPH** (for biosynthetic reactions) and **pentoses** (for nucleotide synthesis).

Key Functions:

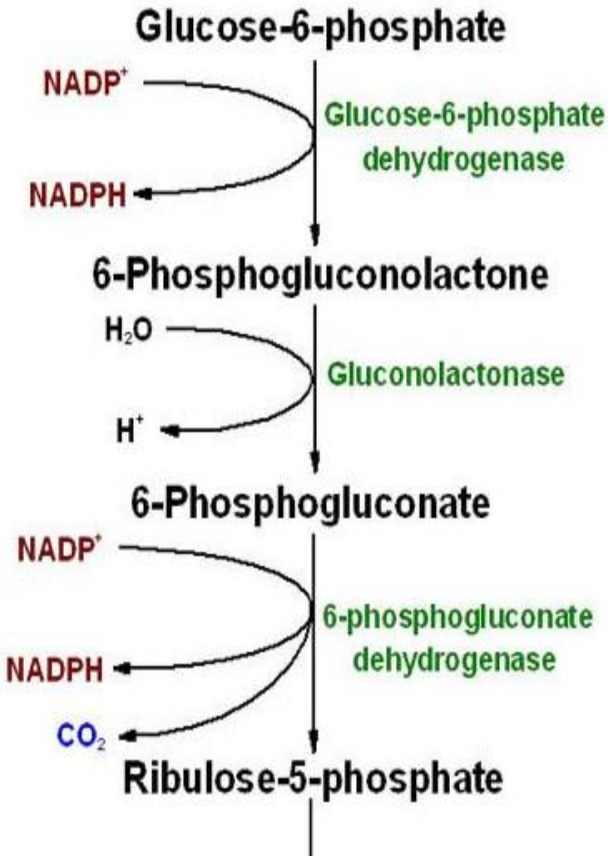
- **Production of NADPH**: Essential for anabolic (biosynthetic) reactions, such as **fatty acid synthesis** and **antioxidant defense**.
- **Production of Ribose-5-Phosphate**: Used for **nucleotide biosynthesis**.

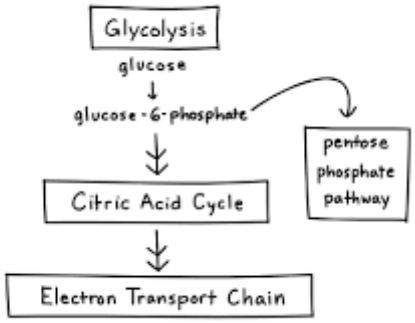
Steps of the HMP Pathway:

1. **Oxidative Phase**:
    - **Glucose-6-phosphate** is oxidized to form **NADPH** and **ribulose-5-phosphate**.
    - NADPH is generated by the reduction of **NADP+**.
2. **Non-Oxidative Phase**:
    - Ribulose-5-phosphate is converted into **ribose-5-phosphate** and **xylulose-5-phosphate**, which can then be used to generate other sugar intermediates or enter glycolysis.

Example:

- **Bacteria**: E. coli utilizes the HMP pathway for the synthesis of nucleotides.
- **Humans**: Red blood cells use the PPP to maintain **glutathione** in its reduced form, protecting against oxidative stress.


Glucose-6-phosphate
NADP+
NADPH
Glucose-6-phosphate dehydrogenase
6-Phosphogluconolactone
H2O
H+
Gluconolactonase
6-Phosphogluconate
NADP+
NADPH
CO2
6-phosphogluconate dehydrogenase
Ribulose-5-phosphate


Glycolysis
glucose
glucose-6-phosphate
pentose phosphate pathway
Citric Acid Cycle
Electron Transport Chain

3. Entner-Doudoroff (ED) Pathway

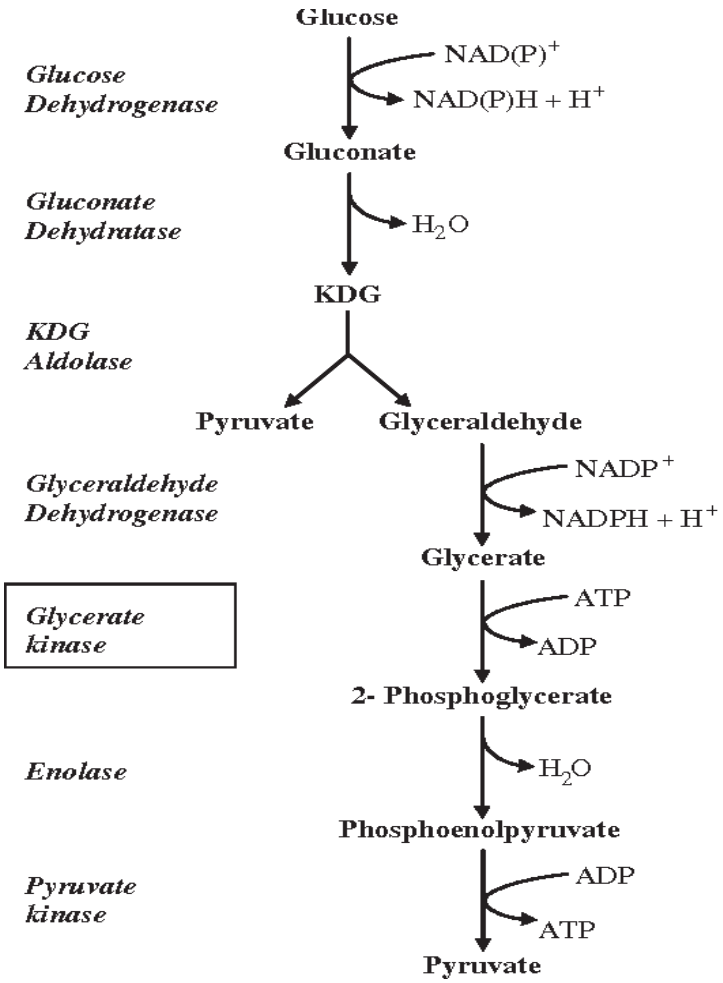
- The **Entner-Doudoroff (ED)** pathway is an alternative glycolytic pathway primarily found in some **bacteria** and **archaea**. It is less efficient than the EMP pathway but still enables energy production from glucose.

Steps of ED Pathway:

1. **Glucose Conversion**: Glucose is oxidized to produce **gluconate**, which is then converted to **2-keto-3-deoxy-6-phosphogluconate (KDPG)**.
2. **Cleavage**: KDPG is cleaved into **pyruvate** and **glyceraldehyde-3-phosphate (G3P)**.
3. **ATP Generation**: G3P enters the glycolytic pathway, and the net result is the production of **1 ATP**, **1 NADH**, and **1 NADPH**.

Example:

- **Bacteria**: The ED pathway is used by bacteria like **Zymomonas mobilis** and some strains of **Pseudomonas** to ferment glucose.



4. Tricarboxylic Acid (TCA) Cycle (Krebs Cycle / Citric Acid Cycle)

- The **TCA cycle** or **Krebs cycle** is a central metabolic pathway in aerobic organisms that plays a key role in energy production through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins.
- **Location**: Occurs in the **mitochondria** of eukaryotes and the **cytoplasm** of prokaryotes.

Steps of the TCA Cycle:

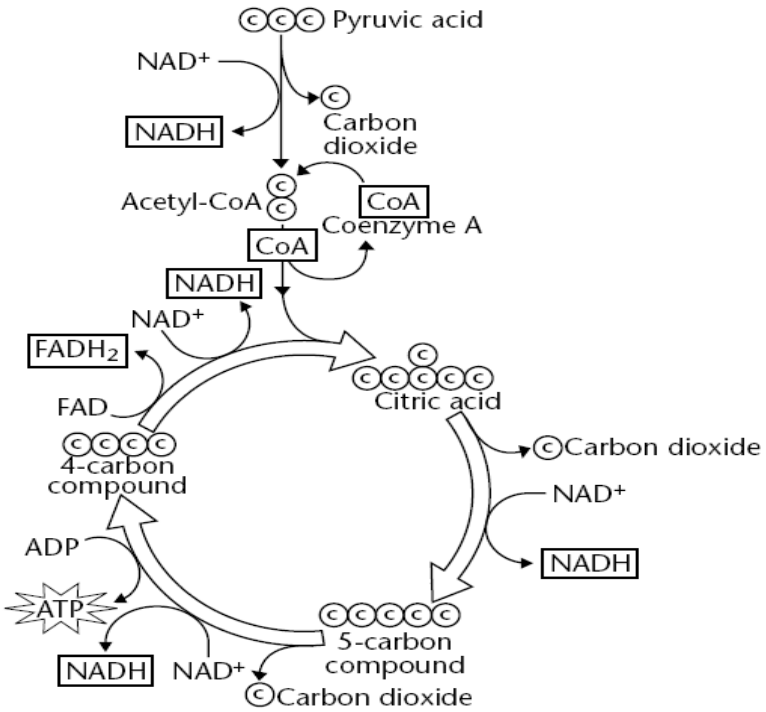
1. **Formation of Acetyl-CoA**: Glucose is broken down into **pyruvate** (via glycolysis), which is then converted into **acetyl-CoA**.
2. **Citrate Formation**: Acetyl-CoA reacts with **oxaloacetate** to form **citrate** (6-carbon).
3. **Decarboxylation and Oxidation**:
    - Citrate is decarboxylated (CO_2 removed) and oxidized, releasing **NADH** and **FADH2**.
4. **Regeneration of Oxaloacetate**: The cycle regenerates **oxaloacetate**, which can react with a new acetyl-CoA to repeat the cycle.

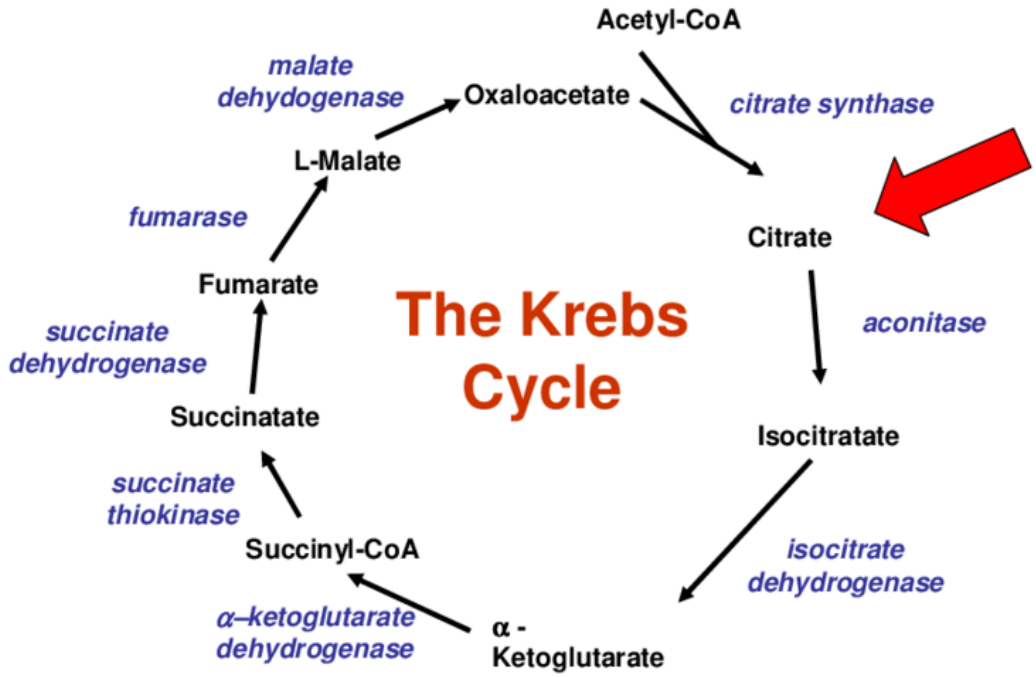
Key Products of the TCA Cycle:

- **3 NADH**
- **1 FADH2**
- **1 ATP (GTP)**
- **2 CO_2** (waste products)

Example:

- **Humans**: The TCA cycle occurs in the **mitochondria** of cells, contributing to the production of ATP and high-energy electron carriers (NADH and FADH2).
- **Bacteria**: Prokaryotic organisms like **E. coli** use the TCA cycle to generate energy under aerobic conditions.


Pyruvic acid
NAD+
NADH
Carbon dioxide
Acetyl-CoA
CoA
Coenzyme A
CoA
NADH
NAD+
FADH2
FAD
Citric acid
4-carbon compound
Carbon dioxide
NAD+
NADH
ADP
ATP
NADH
NAD+
5-carbon compound
Carbon dioxide


Acetyl-CoA
malate dehydogenase
Oxaloacetate
citrate synthase
L-Malate
fumarase
Citrate
Fumarate
The Krebs Cycle
aconitase
succinate dehydrogenase
Succinatate
Isocitratate
succinate thiokinase
isocitrate dehydrogenase
Succinyl-CoA
α–ketoglutarate dehydrogenase
α - Ketoglutarate

Summary Table: Key Glycolytic Pathways

Pathway	Key Features	End Products	Example Organisms
EMP (Glycolysis)	10 steps; net production of 2 ATP and 2 NADH per glucose	2 Pyruvate, 2 ATP (net), 2 NADH	Humans, yeast, E. coli, all organisms
HMP (Pentose Phosphate)	Produces NADPH and ribose-5-phosphate for biosynthesis	NADPH, Ribose-5-phosphate	Red blood cells, E. coli, plant cells
ED (Entner-Doudoroff)	Alternative to EMP, less efficient	1 ATP, 1 NADH, 1 NADPH, 2 Pyruvate	Zymomonas mobilis, Pseudomonas spp.
TCA (Citric Acid Cycle)	Aerobic pathway, oxidative metabolism	3 NADH, 1 FADH2, 1 ATP (GTP), 2 CO2	Humans, E. coli (aerobic conditions)

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UNIT: IV Microbial Respiration and Fermentation

Electron Transport System (ETS) and Oxidative Phosphorylation

The Electron Transport System (ETS) is a series of proteins and molecules found in the inner membrane of mitochondria (in eukaryotic cells) and the plasma membrane (in prokaryotic cells).

Function

It transfers electrons from molecules like NADH and $FADH_2$ to oxygen. As electrons pass through the system, energy is released and used to pump protons (H^+ ions) across the membrane. This creates an energy difference (proton gradient) that is used to make ATP (energy currency of the cell).

Components of ETS:

1. **Electron Carriers:**
    - **NADH and $FADH_2$:** Carry electrons from earlier stages of respiration to the ETS.
    - **Cytochromes:** Proteins that transfer electrons step by step.
    - **Ubiquinone (Coenzyme Q):** A molecule that helps transfer electrons between complexes.
    - **Iron-Sulfur Proteins:** Assist in electron movement.
2. **Complexes in ETS:**
    - **Complex I (NADH dehydrogenase):** Accepts electrons from NADH.
    - **Complex II (Succinate dehydrogenase):** Accepts electrons from $FADH_2$.
    - **Complex III (Cytochrome bc1 complex):** Passes electrons to cytochrome c.
    - **Complex IV (Cytochrome c oxidase):** Transfers electrons to oxygen, forming water.
3. **ATP Synthase:**
    - An enzyme that uses the proton gradient created by the ETS to produce ATP from ADP and phosphate.

Examples of ETS:

- In **humans and animals:** ETS takes place in the mitochondria during cellular respiration.
- In **plants:** Occurs in mitochondria for respiration and in chloroplasts during photosynthesis.
- In **bacteria:** ETS is located in the cell membrane and may use other molecules (instead of oxygen) as the final electron acceptor.

Oxidative Phosphorylation

Oxidative phosphorylation is the process where the energy from the electron transport system (ETS) is used to add a phosphate group to ADP to form ATP.

Function:
It produces most of the ATP during cellular respiration by using oxygen as the final electron acceptor.

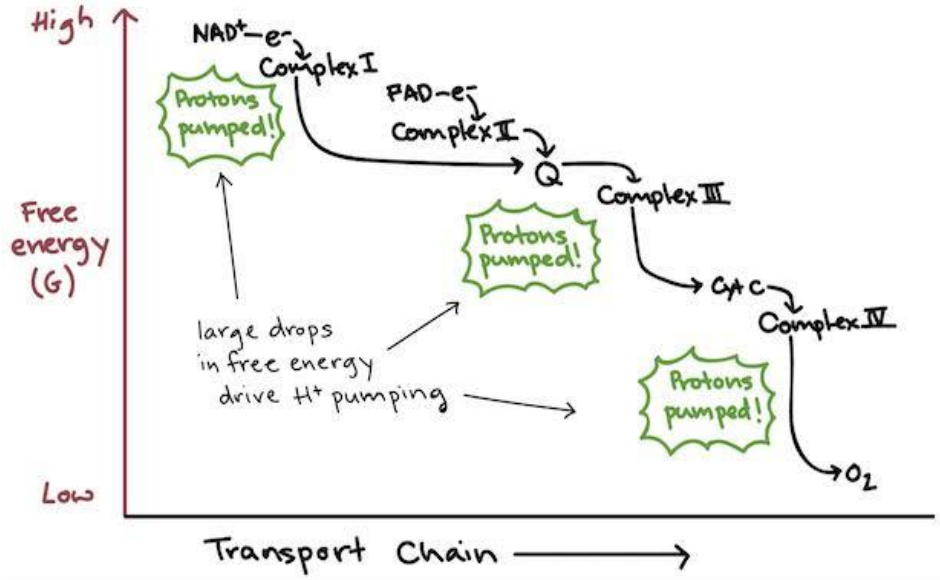
Process of Oxidative Phosphorylation :

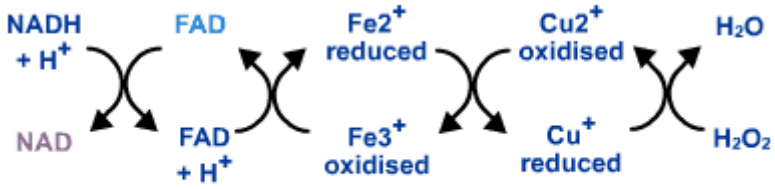
1. **Electron Transfer:**
    - Electrons move through the ETS, releasing energy.
2. **Proton Gradient Formation:**
    - Energy from electrons pumps protons across the membrane, creating a proton gradient.
3. **ATP Production:**
    - Protons flow back through ATP synthase, and this flow drives the production of ATP.

Examples of Oxidative Phosphorylation:

- **In humans:** Occurs in mitochondria during the final stage of aerobic respiration.
- **In plants:** Happens in mitochondria during respiration and in chloroplasts during the light reactions of photosynthesis.
- **In bacteria:** Takes place in the plasma membrane, producing ATP for cellular activities.

Conclusion:
Summary
The ETS and oxidative phosphorylation are critical steps in aerobic respiration that maximize ATP production. Electrons from NADH and FADH2_22 flow through the ETS, creating a proton gradient that drives ATP synthesis via ATP synthase.





3 ATP for each reduced NAD

Anaerobic Respiration: Oxidation of Inorganic Compounds (N, S, Fe, and H)

Anaerobic respiration is a process in which some microorganisms produce energy without using oxygen. Instead, they use other substances like nitrate, sulfate, iron, or carbon dioxide to get energy. In this process, inorganic compounds such as nitrogen (N), sulfur (S), iron (Fe), and hydrogen (H) are oxidized (broken down), which helps in recycling nutrients in nature.

1. Oxidation of Nitrogen (N) Compounds

Microorganisms break down nitrogen compounds like ammonium and nitrite without oxygen. This process helps in recycling nitrogen in the environment.

Nitrification:

Example: *Nitrosomonas* oxidizes ammonia (NH_3) to nitrite (NO_2), and *Nitrobacter* oxidizes nitrite to nitrate (NO_3).

$$NH_3 \rightarrow NO_2^- \rightarrow NO_3$$

Examples :

- *Pseudomonas* and *Paracoccus* (help in removing excess nitrogen).
- *Brocadia* and *Kuenenia* (help in the special process called anammox).

Applications:

- **Wastewater Treatment:** Helps in removing harmful nitrogen compounds from sewage.
- **Soil Fertility:** Keeps nitrogen levels balanced in soil, helping plants grow.
- **Pollution Control:** Reduces nitrogen pollution in water bodies.

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2. Oxidation of Sulfur (S) Compounds

Sulfur compounds like hydrogen sulfide and elemental sulfur are broken down without oxygen. This is important in places where oxygen is not available, such as deep oceans and wetlands.

- **Sulfur Oxidation**:
  - Example: *Thiobacillus* spp. oxidizes hydrogen sulfide (H_2S) or sulfur (S) to sulfate (SO_4).

$$\mathbf{H2S \rightarrow S \rightarrow SO_4^{2-}}$$

Examples:

- *Thiobacillus denitrificans* (breaks down sulfur in water treatment).
- *Beggiatoa* and *Chlorobium* (found in places like hot springs and deep-sea vents).

Applications:

- **Environmental Cleanup:** Helps remove toxic sulfur compounds from polluted areas.
- **Mining:** Assists in extracting metals from ores through bioleaching.
- **Ecosystem Support:** Supports life in extreme environments like deep-sea hydrothermal vents.

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3. Oxidation of Iron (Fe) Compounds

In this process, ferrous iron (Fe^{2+}) is converted to ferric iron (Fe^{3+}) without using oxygen. This process is common in lakes, oceans, and soils where oxygen is low.

Iron Oxidation:

- Example: *Acidithiobacillus ferrooxidans* oxidizes ferrous iron (Fe2+) to ferric iron (Fe3+).

$$Fe2+ \rightarrow Fe3+$$

Examples of Microorganisms:

- *Gallionella* and *Leptothrix* (found in water and soil, help in iron cycling).
- *Acidithiobacillus ferrooxidans* (used in mining to extract metals).

Applications:

- **Mining and Metal Recovery:** Helps recover valuable metals like copper and gold from ores.
- **Water Purification:** Removes iron from groundwater to make it safe for drinking.
- **Soil Formation:** Helps in forming iron-rich layers in soils and rocks.

---

4. Oxidation of Hydrogen (H) Compounds

Hydrogen gas (H_2) is used by some bacteria as an energy source in places without oxygen. The hydrogen is broken down while other substances like carbon dioxide or sulfate are reduced.

Hydrogen Oxidation:

Example: *Hydrogenobacter* spp. oxidizes molecular hydrogen (H2) to produce energy.

$$H2+O2 \rightarrow H2O$$

Examples:

- *Methanobacterium* and *Methanococcus* (produce methane gas in swamps).
- *Desulfovibrio* (reduces sulfate and produces hydrogen sulfide).
- *Acetobacterium woodii* (produces acetic acid).

Applications:

- **Bioenergy Production:** Helps produce biogas (methane).
- **Industrial Processes:** Used in industries to produce chemicals like acetic acid.
- **Supporting Deep-Sea Ecosystems:** Provides energy to organisms living in deep-sea hydrothermal vents.

Conclusion:
Anaerobic respiration involving the oxidation of Nitrogen, Sulfur, Iron, and Hydrogen is essential for life in oxygen-free environments. It plays a major role in recycling nutrients, cleaning up polluted environments, supporting unique ecosystems, and has practical uses in industries like mining, waste management, and energy production.

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FERMENTATION

Fermentation is an anaerobic metabolic process where microorganisms break down organic compounds to generate energy (ATP), without using the electron transport chain or oxygen as the final electron acceptor.

Alcoholic Fermentation

Alcoholic fermentation **is an** anaerobic process **in which** glucose **is broken down into** ethanol (alcohol), carbon dioxide (CO_2), and ATP **without oxygen. This process is carried out by** yeasts (e.g., ***Saccharomyces cerevisiae***) and some bacteria**.**

Pathway:

- Pyruvate from glycolysis is converted into ethanol and carbon dioxide (CO2) via the following steps:
    1. Pyruvate decarboxylation to acetaldehyde (CH_3CHO) and CO_2.
    2. Reduction of acetaldehyde to ethanol by alcohol dehydrogenase.
- **ATP Yield**: 2 ATP per glucose molecule (via glycolysis).
- **Examples**:
- *Saccharomyces cerevisiae* (yeast): Used in brewing and baking.
- *Zymomonas mobilis*: Found in sugar-rich environments like plant saps.

General Reaction:

$$\text{Glucose } (C_6H_{12}O_{6)} \rightarrow \quad 2C_2H_5OH + \quad 2CO_2 + 2ATP$$

Applications of Alcoholic Fermentation

- **Food & Beverage Industry:** Produces **beer, wine, spirits**, and helps dough rise in baking.
- **Biofuel Production:** Ethanol from fermentation is used in biofuels.
- **Pharmaceutical Industry:** Used in **solvents, disinfectants, and antibiotic production**.
- **Industrial & Chemical Uses:** Produces **vinegar** and aids in **biochemical research**.
- **Environmental & Waste Management:** Converts agricultural waste into **bioethanol**, reducing waste.

Lactic Acid Fermentation

Lactic acid fermentation is an **anaerobic metabolic process** in which glucose or other six-carbon sugars are converted into **lactic acid** and ATP, without the use of oxygen. This process is carried out by certain **bacteria (e.g., Lactobacillus)** and **animal cells (e.g., muscle cells)** when oxygen is limited.

General Reaction:

Glucose $(C_6H_{12}O_6)$ → 2Lactic Acid + 2ATP

Types:

1. **Homolactic Fermentation**:
   - Produces only lactic acid.
   - Example: *Lactobacillus* spp., *Streptococcus* spp.
2. **Heterolactic Fermentation**:
   - Produces lactic acid, ethanol, and CO2.
   - Example: *Leuconostoc* spp.

- **ATP Yield**: 2 ATP per glucose molecule (via glycolysis).

Applications of Lactic Acid Fermentation

1. **Food Industry**
   - **Dairy Products:** Produces yogurt, cheese, buttermilk, and kefir (Lactobacillus, *Streptococcus*).
   - **Fermented Vegetables:** Used in sauerkraut, kimchi, and pickles for flavor and preservation.
   - **Beverages:** Produces fermented drinks like kanji and kefir.
2. **Industrial Applications**
   - **Lactic Acid is used in production of** biodegradable plastics (PLA), pharmaceuticals, and cosmetics.

3. **Medical and Health Benefits**

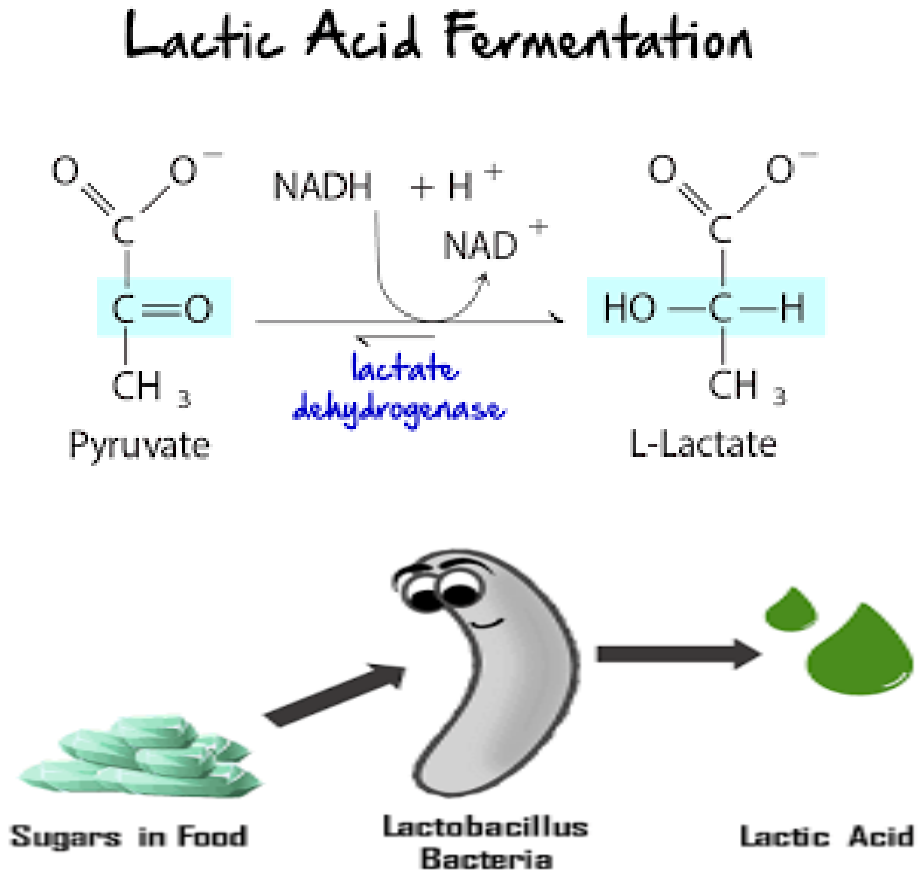
    - **Probiotics:** Supports gut health by promoting beneficial bacteria.
    - **Muscle Energy:** Provides energy during intense exercise when oxygen is low.

4. **Waste Utilization & Sustainability**

    - **Biotechnology:** Converts agricultural waste into valuable organic acids.
    - **Eco-Friendly Plastics:** Produces sustainable, biodegradable plastics.

Summary

- **Alcoholic Fermentation**: Produces ethanol and CO_2, used in brewing and baking (*Saccharomyces cerevisiae*).
- **Lactic Acid Fermentation**: Produces lactic acid, crucial for dairy product fermentation (*Lactobacillus* spp.).



UNIT V: Bacterial Photosynthesis

PHOTOSYNTHETIC PIGMENTS & APPARATUS IN PROKARYOTES

Photosynthetic Pigments

Photosynthetic pigments are **colored molecules** that **absorb sunlight** and help convert it into energy during photosynthesis. Different pigments absorb light of different colors (wavelengths).

Types and Examples of Photosynthetic Pigments:

1. **Chlorophylls:**
   - Absorb sunlight, especially blue and red light, and reflect green light (which is why plants look green).
   - **Examples:**
     - **Chlorophyll a:** Main pigment in all photosynthetic organisms like cyanobacteria.
     - **Chlorophyll b:** Helps in absorbing additional light, found in some bacteria.
2. **Carotenoids:**
   - Absorb blue and green light, reflecting yellow, orange, and red colors. They also protect cells from damage by sunlight.
   - **Examples:**
     - **Beta-carotene:** Gives carrots their orange color.
     - **Lutein:** Found in many green plants and algae.
3. **Phycobilins:**
   - Absorb green, yellow, and orange light, allowing photosynthesis in deeper or shaded water where light is limited.
   - **Examples:**
     - **Phycocyanin:** Blue pigment found in cyanobacteria.
     - **Phycoerythrin:** Red pigment found in red algae and some cyanobacteria.
4. **Bacteriochlorophylls**:
   - Found in anoxygenic photosynthetic bacteria.
   - Absorb light in different spectra compared to chlorophyll a.

Importance of Photosynthetic Pigments:

- Capture sunlight for energy production.
- Help organisms survive in different light conditions.
- Give color to organisms (like green plants or red algae).

Photosynthetic Apparatus in Prokaryotes

The **photosynthetic apparatus** in prokaryotes is the set of structures and molecules that work together to carry out photosynthesis. It includes pigments, membranes, and proteins needed to capture light and convert it into energy.

Components of the Photosynthetic Apparatus:

1. **Thylakoid Membranes:**
    - Flattened sacs where photosynthetic pigments and proteins are located.
    - It is the site where light energy is absorbed and converted into chemical energy.
    - **Example:** In cyanobacteria, thylakoid membranes float freely in the cytoplasm.
2. **Photosystems (PSI and PSII):**
    - Groups of pigments and proteins that work together to capture light energy.
    - They pass the absorbed light energy to the electron transport system for energy production.
    - **Example:** Cyanobacteria have both PSI and PSII, similar to plants.
3. **Reaction Centers:**
    - Special molecules where the actual conversion of light energy into chemical energy happens.
    - **Example:** Bacteria like *Rhodobacter* use a single reaction center in their photosynthetic process.
4. **Electron Transport System (ETS):**
    - **Function:** Transfers energy from the reaction centers to make ATP (energy) and NADPH (used in making food).

Examples of Prokaryotic Photosynthetic Apparatus

1. **Cyanobacteria**:
    - Contain chlorophyll a and phycobilins.
    - Conduct oxygenic photosynthesis.
    - Example: *Anabaena*, *Nostoc*.
2. **Purple Sulfur Bacteria**:
    - Use bacteriochlorophyll in vesicles.
    - Conduct anoxygenic photosynthesis using hydrogen sulfide.
    - Example: *Chromatium*.
3. **Green Sulfur Bacteria**:
    - Contain chlorosomes rich in bacteriochlorophyll.
    - Conduct anoxygenic photosynthesis.
    - Example: *Chlorobium*.

Conclusion:

Photosynthetic pigments and the photosynthetic apparatus in prokaryotes are essential for capturing sunlight and converting it into usable energy.These processes are vital for life, supporting ecosystems, producing oxygen, and offering solutions for sustainable energy.

OUTLINE OF OXYGENIC PHOTOSYNTHESIS IN BACTERIA

Oxygenic photosynthesis is the process by which certain bacteria, like cyanobacteria, use sunlight, water, and carbon dioxide to make their own food (glucose) and release oxygen as a byproduct. This process is similar to how plants perform photosynthesis.

Components Involved:

1. **Light Source:**
    - Sunlight provides energy for the process.
2. **Pigments:**
    - **Chlorophyll a:** The main pigment that absorbs light.
    - **Accessory pigments (e.g., phycobilins):** Help capture more light energy.
3. **Photosystems:**
    - **Photosystem II (PSII):** Captures light and splits water molecules.
    - **Photosystem I (PSI):** Helps form energy-rich molecules like NADPH.
4. **Electron Transport Chain (ETC):**
    - A series of proteins that transfer electrons, releasing energy to form ATP.
5. **Water (H_2O):**
    - The source of electrons and protons; oxygen is released when water is split.
6. **Carbon Dioxide (CO_2):**
    - Used to make glucose during the Calvin cycle.
7. **Energy Carriers:**
    - **ATP (Adenosine Triphosphate):** Stores energy for cell processes.
    - **NADPH:** Carries electrons and hydrogen for making glucose.

Procedure (Steps of Oxygenic Photosynthesis):

1. **Light Absorption:**
    - Sunlight hits chlorophyll in Photosystem II.

    - Energy excites electrons, which are passed down the electron transport chain.
2. **Water Splitting (Photolysis):**
    - Water molecules split into oxygen, protons, and electrons.
    - Oxygen is released into the environment.
3. **ATP Formation:**
    - As electrons move through the electron transport chain, energy is used to make ATP.
4. **NADPH Formation:**
    - In Photosystem I, sunlight excites electrons again.
    - These electrons help form NADPH.
5. **Carbon Fixation (Calvin Cycle):**
    - ATP and NADPH are used to convert carbon dioxide into glucose.
    - This cycle does not require light (light-independent reactions).

Examples of Oxygenic Photosynthetic Bacteria

Cyanobacteria

1. *Anabaena*:
    - Nitrogen-fixing cyanobacteria with specialized cells (heterocysts).
2. *Synechococcus*:
    - Marine cyanobacteria contributing significantly to global oxygen production.
3. *Prochlorococcus*:
    - Tiny cyanobacteria, responsible for a large fraction of photosynthesis in oceans.

Summary

Oxygenic photosynthesis releases oxygen.
It helps convert solar energy into chemical energy.
Cyanobacteria played a major role in increasing oxygen levels in Earth's atmosphere

OUTLINE OF ANOXYGENIC PHOTOSYNTHESIS IN BACTERIA

Anoxygenic photosynthesis is a form of photosynthesis that does not produce oxygen as a byproduct.
It uses electron donors other than water, such as hydrogen sulfide (H2S), organic compounds, or ferrous ions (Fe2+).
Common in certain groups of photosynthetic bacteria like **purple bacteria**, **green sulfur bacteria**, and **heliobacteria**.

Components Involved:

1. **Light Source:**
   - Sunlight provides energy for photosynthesis.
2. **Pigments:**
   - **Bacteriochlorophyll:** The main pigment that absorbs light, different from the chlorophyll in plants.
   - **Carotenoids:** Accessory pigments that help absorb light and protect cells from damage.
3. **Photosystem:**
   - Usually only **Photosystem I (PSI)** is present (unlike oxygenic photosynthesis which uses PSI and PSII).
4. **Electron Donors:**
   - Substances other than water provide electrons. Common examples:
     - **Hydrogen sulfide (H_2S)**
     - **Hydrogen gas (H_2)**
     - **Iron (Fe^{2+})**
     - **Organic acids**
5. **Electron Transport Chain (ETC):**
   - Transfers electrons and helps form ATP.
6. **Carbon Dioxide (CO_2):**
   - Used to make glucose in the Calvin cycle or other similar pathways.
7. **Energy Carriers:**
   - **ATP (Adenosine Triphosphate):** Stores energy for cell activities.
   - **NADPH/NADH:** Carries electrons for sugar production.

Procedure (Steps of Anoxygenic Photosynthesis):

1. **Light Absorption:**
   - Sunlight is absorbed by bacteriochlorophyll in the photosystem.
2. **Electron Donation:**

- Instead of splitting water, the bacteria take electrons from substances like hydrogen sulfide (H_2S).
- Example: $H_2S \rightarrow 2H^+ + 2e^- + S$ (sulfur is often released instead of oxygen).

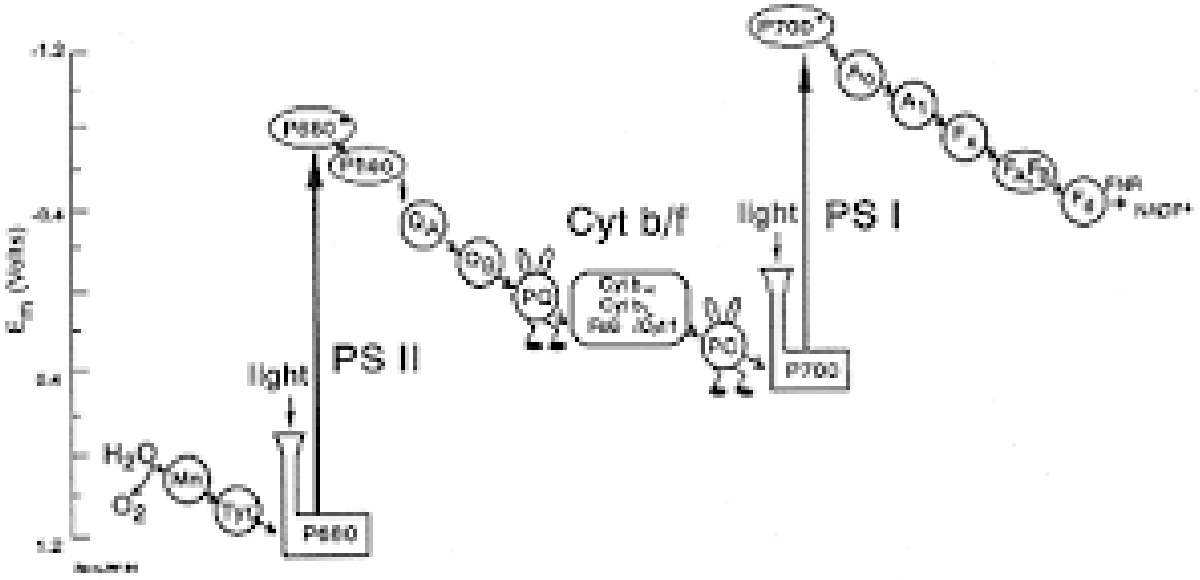
3. **ATP Formation:**
   - As electrons move through the electron transport chain, energy is released and used to form ATP.
4. **NADPH/NADH Formation:**
   - Electrons eventually help form NADPH or NADH, needed for glucose production.
5. **Carbon Fixation:**
   - ATP and NADPH are used in the Calvin cycle (or other pathways) to convert CO_2 into glucose.

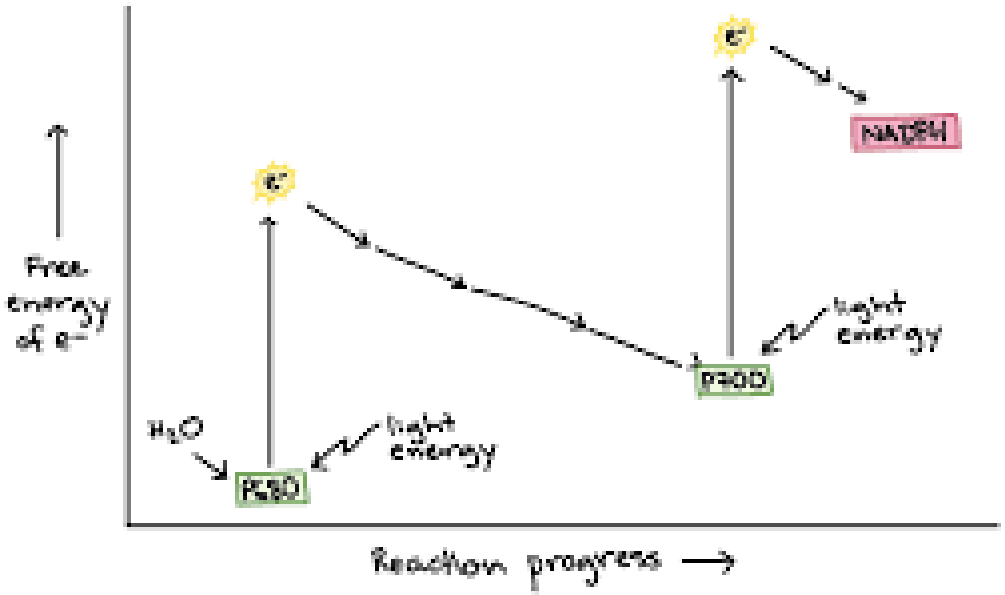
Examples of Anoxygenic Photosynthetic Bacteria

1. **Purple Sulfur Bacteria**
   - Use H2S as an electron donor and deposit elemental sulfur.
   - Example: *Chromatium*.
2. **Green Sulfur Bacteria**
   - Use H2S and deposit sulfur extracellularly.
   - Example: *Chlorobium*.
3. **Purple Non-Sulfur Bacteria**
   - Use organic molecules as electron donors.
   - Example: *Rhodospirillum*.
4. **Heliobacteria**
   - Perform photosynthesis under anaerobic conditions.
   - Example: *Heliobacterium*.
5. **Filamentous Anoxygenic Phototrophs**
   - Thrive in low-oxygen aquatic environments.
   - Example: *Chloroflexus*.

Summary:

- Anoxygenic photosynthesis does **not** release oxygen.
- Uses only **Photosystem I** (PSI).
- Occurs in environments where oxygen is low or absent.
- Important for recycling sulfur and other elements in nature.


Cyt b/f
light
PS II
light
PS I
P680
P700
H_2O
O_2


e⁻
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NADPH
Free energy of e⁻
light energy
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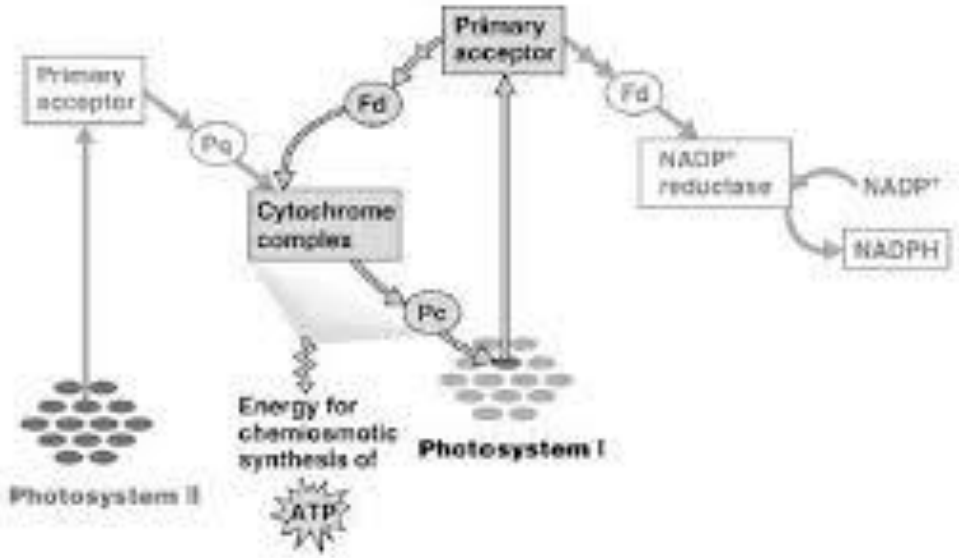
H_2O
light energy
P680
Reaction progress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Pq
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Primary acceptor
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NADP+ reductase
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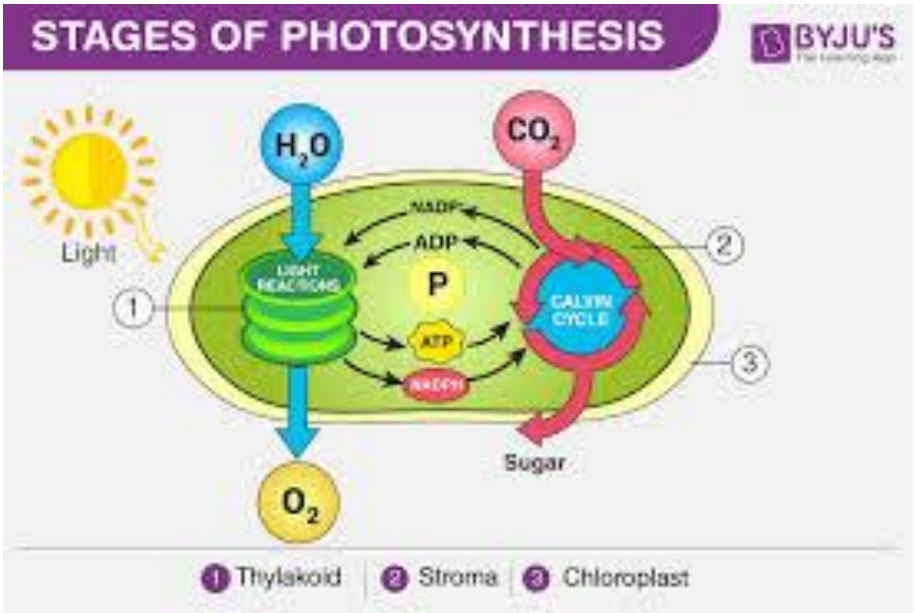
NADPH
Cytochrome complex
Pc
Energy for chemosmotic synthesis of
ATP
Photosystem II
Photosystem I


STAGES OF PHOTOSYNTHESIS
BYJU'S
Light
H_2O
CO_2
NADP+
ADP
P
ATP
NADPH
LIGHT REACTIONS
CALVIN CYCLE
1
2
3
Sugar
O_2
1 Thylakoid
2 Stroma
3 Chloroplast

IV Semester, MINOR **C 10 (- MICROBIAL PHYSIOLOGY AND METABOLISM)**

Time : 3Hrs Max Marks : 50

SECTION – A

Answer all the following questions, Draw labelled diagrams wherever necessary (5x8=40 Marks)

UNIT	Q.No.	Questions	Marks
I	1.	Explain Nutritional requirements of Microorganisms	8
	2	Describe Nutritional groups of microorganisms-based on C, energy and electron sources	
	3	Explain Growth media - synthetic, nonsynthetic, selective, enrichment and differential media	8
II	1.	Explain different phases of growth in batch cultures.	8
	2	Write notes on Factors influencing microbial growth	
	3	Describe Methods for measuring microbial growth - Direct microscopy, viable count estimates,turbidometry and biomass	8
III	1	Explain First and Second law of Thermodynamics. Open and Closed system.	8
	2	Write about the Structure and Function of NAD and FAD.	
	3	Explain Glycolytic pathway and mention its significance.	
IV	1	Explain ETS and oxidative phosphorylation.	
	2	Briefly anaerobic respiration.	
	3	Explain Fermentative modes in microorganisms with special reference to alcoholic, Lactic acid fermentations	
V	1	Explain Photosynthetic pigments, Photosynthetic apparatus in prokaryotes	8
	2	Explain Outline of oxygenic photosynthesis in bacteria	8
	3	Explain Outline of anoxygenic photosynthesis in bacteria	

Section -B

Answer any five of the following

(10x1=10)

UNIT-1

1. Photoautotrophs obtain both their energy and carbon from organic compounds.

(False)

2. Facilitated diffusion requires ATP to transport molecules across the membrane.

(False)

3. Iron uptake in microorganisms often involves siderophores to help transport iron into the cell. (True)

4. _____ is a transport process that moves molecules against their concentration gradient using ATP. (Primary active transport)

5. Microorganisms that use organic carbon as their carbon source are called _____. (Heterotrophs)

UNIT-2

1. The lag phase in batch culture is a period of intense cell division. (False)

2. Generation time is the time required for a microbial population to double. (True)

3. Turbidometry is a method used to directly count individual microbial cells.

(False)

4. The time required for a microbial population to double in number is called _____. (Generation time)

5. In direct microscopic counting, a special slide called a _____ chamber is

often used. (Petroff-Hausser or Hemocytometer)

UNIT-3

1. The first law of thermodynamics states that energy can be created and destroyed.

(False)

2. ATP is considered a high-energy molecule due to its phosphoanhydride bonds.

(True)

3. The _____ law of thermodynamics states that entropy of a closed system

always increases over time. (Second)

4. The standard free energy change of ATP hydrolysis is approximately _____

kcal/mol. (-7.3 kcal/mol)

5. The _____ cycle is a central metabolic pathway that generates NADH

and
$FADH_2$ for oxidative phosphorylation. (TCA/Krebs)

UNIT-4
1. Oxidative phosphorylation generates ATP using a proton gradient established by
the ETS. (True)
2. Chemoautotrophs obtain their energy by oxidizing inorganic compounds such as
ammonia or hydrogen sulfide. (True)
3. Lactic acid fermentation produces ethanol and carbon dioxide as major byproducts. (False) (It produces lactic acid, not ethanol.)
4. _____ is the final electron acceptor in aerobic respiration. (Oxygen / O_2)
5. In anaerobic respiration, microorganisms use electron acceptors such as _____ instead of oxygen. (Nitrate / Sulfate / CO_2 / Ferric ions)

UNIT-5
1. Chlorophyll a is the only photosynthetic pigment found in all photosynthetic bacteria. (False)
2. The photosynthetic apparatus in prokaryotes is located in the plasma membrane or
specialized membrane structures. (True)
3. Anoxygenic photosynthesis uses water as an electron donor, similar to oxygenic
photosynthesis. (False)
4. The _____ is the structure in prokaryotes where photosynthesis takes place.
(Photosynthetic apparatus)
5. In oxygenic photosynthesis, water is split to generate electrons, releasing _____ as a byproduct. (Oxygen/O_2)