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MCB424

MICROBIAL PHYSIOLOGY & METABOLISM

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Course Code: MCB 424
Course Title: Microbial Physiology & Metabolism
Course Unit: 3 Units

Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems
Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors
Series 3: Biochemistry of Microbial Processes and Biosynthesis
Series 4: Bioenergetics, Enzyme Action, and Metabolic Pathways
Series 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Series 1 Bacterial Anatomy, Growth Dynamics, and Culture Systems

Series outline

- **Part 1: Bacterial Anatomy and Cytochemistry**

- 1.1: Structural organisation of bacterial cells
- 1.2: Cytochemical composition and functions

- **Part 2: Growth Dynamics of Bacterial Cultures**

- 2.1: Batch culture growth phases
- 2.2: Continuous culture systems
- 2.3: Mathematical modelling of growth

- **Part 3: Factors Affecting Bacterial Growth**

- 3.1: Physical factors (temperature, pH, oxygen, etc.)
- 3.2: Chemical factors (nutrients, inhibitors, toxins)

- **Part 4: Culture Systems and Applications**

- 4.1: Laboratory culture techniques
- 4.2: Industrial and research applications

Introduction

Micro-organisms are among the most diverse and adaptable forms of life, and bacteria in particular play a central role in both natural ecosystems and industrial applications. To study their physiology and metabolism effectively, it is essential to first understand their structural organisation, how they grow under different conditions, and the systems used to culture them. This Series provides the foundation for appreciating bacterial life processes, linking cellular anatomy with growth behaviour and practical culture methods.
The aim of this Series is to equip you with the knowledge and skills to describe bacterial anatomy, analyse growth dynamics, evaluate the influence of environmental factors, and apply culture systems in both laboratory and industrial contexts.

We shall commence this Series by examining bacterial anatomy and cytochemistry, focusing on the structural organisation and chemical composition of bacterial cells. Next, we will explore the dynamics of bacterial growth, considering both batch and continuous culture systems as well as mathematical modelling of growth. Following that, we will analyse the physical and chemical factors that affect bacterial growth, such as temperature, pH, nutrients, and inhibitors. Finally, we will conclude by studying culture systems and their applications, including laboratory techniques and industrial uses. This sequential approach will help you build a clear and connected understanding of bacterial physiology from structure to application.

Got it, thank you for pointing that out. Since this is a **3-unit course**, each Series should feel substantial, with **3 to 5 well-developed learning outcomes** that are specific, measurable, and aligned with the Parts. Let me expand the **Series Learning Outcomes (SLOs)** for **Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems** so they are more comprehensive and detailed, while still clear and learner-focused.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **1.1** define the structural organisation of bacterial cells and describe the functions of their cytochemical components,
- **1.2** explain how bacterial anatomy supports physiological processes such as transport and respiration,
- **1.3** analyse the growth phases of bacterial cultures in batch systems and interpret growth curves,
- **1.4** evaluate continuous culture systems by comparing their design, operation, and advantages over batch cultures,
- **1.5** calculate growth parameters using mathematical models and apply them to predict bacterial population dynamics,
- **1.6** assess the influence of physical factors such as temperature, pH, osmotic pressure, and oxygen availability on bacterial growth,
- **1.7** examine the impact of chemical factors including nutrient composition, inhibitors, and toxins on bacterial growth,
- **1.8** demonstrate laboratory culture techniques for isolating and maintaining bacterial populations,
- **1.9** apply knowledge of culture systems to evaluate industrial and research applications of bacterial growth.

1.1 Bacterial Anatomy And Cytochemistry

1.1.1 Structural organisation of bacterial cells

Bacteria are single-celled micro-organisms that exhibit remarkable adaptability due to their structural simplicity and efficiency. The **cell wall** is a rigid layer that provides shape and protection, primarily composed of **peptidoglycan**, a polymer of sugars and amino acids. The **cell membrane** lies beneath the wall and regulates the transport of substances in and out of the cell. Inside, the **cytoplasm** contains enzymes, nutrients, and ribosomes, which are responsible for protein synthesis. Unlike eukaryotic cells, bacteria lack a true nucleus; instead, their genetic

material is located in the **nucleoid**, a region containing a single circular DNA molecule. Many bacteria also possess **plasmids**, which are small DNA fragments that carry additional genes, often related to antibiotic resistance.
Some bacteria have specialised structures such as **flagella**, which are whip-like appendages used for motility, and **pili**, which are hair-like projections that aid in attachment and genetic exchange. The **capsule**, a polysaccharide layer surrounding some bacterial cells, enhances protection against host immune systems.
Fill in the blank: The rigid layer that provides shape and protection in bacterial cells is called the _____.

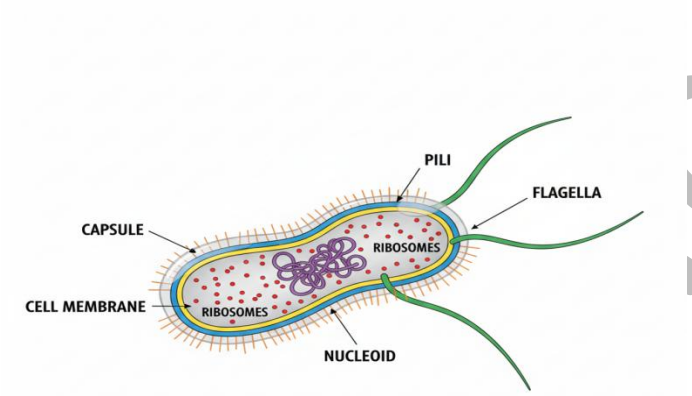

Figure 1.1 Structural organisation of a bacterial cell

1.1.2 Cytochemical composition and functions

The cytochemical composition of bacteria reflects their metabolic versatility. The **cytoplasm** is rich in proteins, enzymes, and metabolites that drive biochemical reactions. **Ribosomes** are abundant and serve as the sites of protein synthesis, translating genetic information into functional proteins. The **DNA** in the nucleoid encodes essential genes for survival, while **RNA molecules** act as intermediaries in protein production.
Bacteria store energy in the form of **inclusion bodies**, which may contain glycogen, polyphosphate, or lipids. These reserves allow bacteria to survive in nutrient-limited environments. The **cell wall** not only provides structural support but also plays a role in determining Gram-positive or Gram-negative classification, which is based on differences in peptidoglycan thickness and outer membrane presence.
Fill in the blank: The cytoplasmic structures that serve as sites of protein synthesis are called _____.

Component	Primary Function
Cell Wall	Provides structural support, protection, and maintains the cell's shape; prevents osmotic lysis.

Component	Primary Function
Cell Membrane	Regulates the transport of nutrients and waste; site of metabolic processes like respiration in some species.
Cytoplasm	The gel-like matrix that houses the internal structures and serves as the site for most metabolic reactions.
Ribosomes	The protein factories of the cell; they translate genetic code into functional proteins.
Nucleoid	The region containing the primary bacterial chromosome (DNA); controls cell genetics and reproduction.
Plasmids	Small, circular extrachromosomal DNA; often carry "bonus" genes like antibiotic resistance.
Capsule	A protective outer layer that helps the cell evade the host's immune system and prevents dehydration.
Flagella	Tail-like structures that provide motility, allowing the bacteria to move toward nutrients or away from toxins.
Pili (Fimbriae)	Hair-like appendages used for attachment to surfaces or for the transfer of DNA between cells (conjugation).

Table 1.1 Cytochemical components of bacterial cells and their functions

Pilot 1.1

1. The gel-like substance inside the bacterial cell where metabolic reactions occur is called _____.
2. The structure responsible for bacterial motility is the _____.
3. The protective outer layer found in some bacteria that helps evade host defences is the _____.
4. The macromolecule that stores genetic information in bacterial cells is _____.
5. The primary structural component of bacterial cell walls is _____.
6. The molecule that serves as the main energy currency in bacterial cells is _____.

1.2 Growth Dynamics Of Bacterial Cultures

1.2.1 Batch culture growth phases

When bacteria are grown in a closed system with a fixed amount of nutrients, they exhibit distinct **growth phases**. These include the **lag phase**, where cells adapt to their environment and

prepare for division, the **log phase**, characterised by rapid exponential growth, the **stationary phase**, where nutrient depletion and waste accumulation slow growth, and the **death phase**, where cells decline due to unfavourable conditions. Each phase reflects changes in metabolic activity and population size.

Fill in the blank: The phase in which bacteria grow at their maximum rate is called the _____ phase.

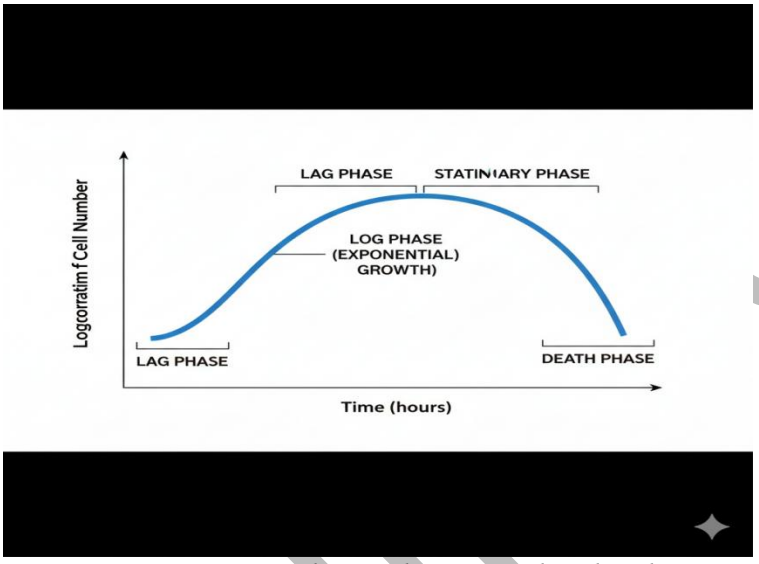

Figure 1.2 Bacterial growth curve in batch culture

1.2.2 Continuous culture systems

Unlike batch cultures, **continuous culture systems** maintain bacterial growth by constantly supplying fresh nutrients and removing waste products. The most common system is the **chemostat**, which regulates growth rate by controlling nutrient flow. This allows bacteria to remain in the log phase for extended periods, making continuous cultures valuable for industrial microbiology and research.

Fill in the blank: A continuous culture system that maintains bacterial growth by regulating nutrient flow is called a _____.

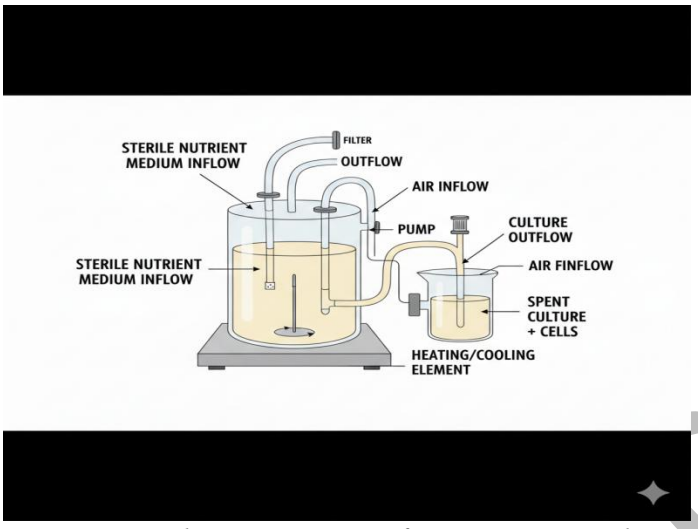

Figure 1.3 Chemostat system for continuous culture

1.2.3 Mathematical modelling of growth

To quantify bacterial growth, scientists use **mathematical models**. The most common is the exponential growth equation:

[N_t = N_0 \cdot e^{\mu t}]

where (N_t) is the population at time (t), (N_0) is the initial population, (\mu) is the specific growth rate, and (e) is the base of natural logarithms. These models help predict population dynamics, optimise culture conditions, and design industrial processes.

Fill in the blank: In the exponential growth equation, the symbol (\mu) represents the _____ growth rate.

Bacterial growth typically follows a predictable pattern where the population doubles at a constant rate during the exponential (log) phase. To quantify this, we use several key equations to relate the initial population to the final population over a specific period of time.

Box 1.1 Key equations in bacterial growth modelling

Parameter	Definition	Units (Typical)
N_t	Population size at time t	Cells or CFU/mL
N_0	Initial population size	Cells or CFU/mL
n	Number of generations (doublings)	Unitless
t	Total time of elapsed growth	min, hr, or days

Parameter	Definition	Units (Typical)
g (or G)	Generation time (doubling time)	time per generation
μ	Specific growth rate constant	$time^{-1}$

Core Equations

1. The Exponential Growth Equation

The fundamental relationship showing that the population increases by a power of 2 for every generation n:

$$N_t = N_0 \times 2^n$$

2. Calculating Generations (n)

To find how many times the population doubled, we use logarithms (typically $\log_{10}$ or $\ln$):

$$n = \frac{\log N_t - \log N_0}{\log 2} \approx \frac{\log N_t - \log N_0}{0.301}$$

3. Generation Time (g)

The average time it takes for a population to double:

$$g = \frac{t}{n}$$

4. Specific Growth Rate (μ)

The rate of change of the bacterial population per unit of binary fission:

$$\mu = \frac{\ln N_t - \ln N_0}{t}$$

(Note: Relationship between μ and g is $\mu = \frac{\ln 2}{g} \approx \frac{0.693}{g}$)

Pilot 1.2

1. The initial phase where bacteria adjust to a new environment before dividing is the _____ phase.
2. The phase of bacterial growth where cells divide rapidly is the _____ phase.
3. The phase where nutrient depletion slows growth and cell death balances cell division is the _____ phase.
4. A system that maintains bacterial growth at a steady state by continuously adding fresh medium is called a _____.
5. The apparatus commonly used to maintain continuous culture conditions is the _____.
6. The mathematical expression that describes bacterial growth rate is called the _____ equation.
7. The parameter that represents the maximum growth rate of bacteria is denoted by _____.

1.3 Factors Affecting Bacterial Growth

1.3.1 Physical factors

Bacterial growth is strongly influenced by **physical factors**, which include temperature, pH, oxygen availability, osmotic pressure, and light. Each bacterial species has an optimal range for these conditions. For example, **psychrophiles** thrive at low temperatures, **mesophiles** grow best at moderate temperatures, and **thermophiles** prefer high temperatures. Similarly, bacteria can be classified based on their oxygen requirements: **aerobes** require oxygen, **anaerobes** grow without oxygen, and **facultative anaerobes** can adapt to both conditions.

The pH of the environment also plays a crucial role. **Acidophiles** grow in acidic conditions, while **alkaliphiles** prefer alkaline environments. Osmotic pressure, influenced by salt concentration, determines whether bacteria can survive in high-salt environments, as seen in **halophiles**.

Fill in the blank: Bacteria that thrive in high-temperature environments are called _____.

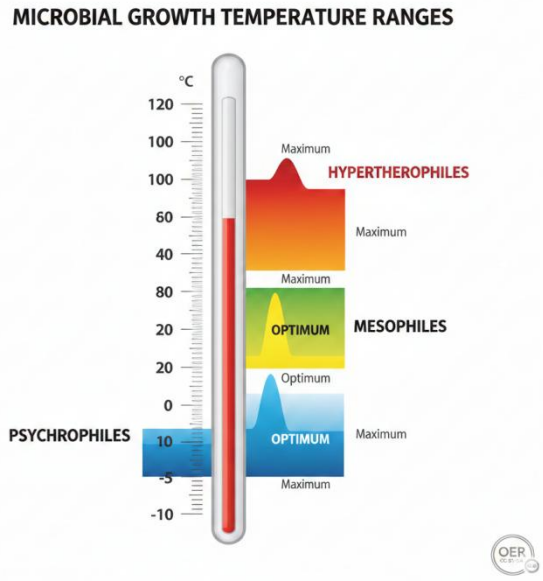

Figure 1.4 Temperature ranges for bacterial growth

1.3.2 Chemical factors

In addition to physical conditions, **chemical factors** such as nutrient composition, inhibitors, and toxins significantly affect bacterial growth. Nutrients like carbon, nitrogen, sulphur, and phosphorus are essential for biosynthesis and energy metabolism. The availability of trace elements such as iron and magnesium also influences enzymatic activity.

Growth can be inhibited by the presence of toxic substances, including heavy metals or antibiotics. Some bacteria develop resistance mechanisms, such as efflux pumps or enzyme production, to counteract these inhibitors. The balance between nutrient supply and inhibitory compounds determines whether bacteria will flourish or decline.

Fill in the blank: The essential nutrient required for protein synthesis and enzyme activity is _____.

Essential Nutrients for Bacterial Growth

Nutrients are generally categorized into **macronutrients** (needed in large amounts) and **micronutrients** or **trace elements** (needed in tiny amounts).

Category	Nutrient	Biological Role
Macronutrients	**Carbon**	The structural backbone of all organic molecules.
	Nitrogen	Essential for synthesis of proteins and nucleic acids (DNA/RNA).
	Sulfur	Required for specific amino acids (cysteine, methionine) and vitamins.
	Phosphorus	Critical for ATP, phospholipids, and nucleic acids.
Micronutrients	**Iron**	A key cofactor for enzymes and the electron transport chain.
	Trace Elements	Ions like Zn^{2+}, Cu^{2+}, and Mn^{2+} that act as enzyme cofactors.
Growth Factors	**Vitamins/Amino Acids**	Organic compounds some bacteria cannot synthesize on their own.

Common Growth Inhibitors

Inhibitors can occur naturally or be introduced (like antibiotics or disinfectants) to control microbial growth.

Type of Inhibitor	Mechanism of Action	Example
Heavy Metals	Denature proteins and disrupt enzyme function.	Silver (Ag), Mercury (Hg), Lead (Pb)
Antibiotics	Specifically target bacterial structures (cell walls, ribosomes).	Penicillin, Tetracycline
Oxidizing Agents	Damage cellular components through free radicals.	Hydrogen peroxide, Chlorine/Bleach
Alcohols	Dissolve lipid membranes and coagulate proteins.	Ethanol, Isopropanol

Type of Inhibitor	Mechanism of Action	Example
High Osmotic Pressure	Causes plasmolysis (cell shrinking) by drawing water out.	High salt or sugar concentrations
Extreme pH	Disrupts ionic bonds in proteins and interferes with H^+ gradients.	Strong acids or bases

Table 1.2 Essential chemical factors influencing bacterial growth

Pilot 1.3

1. Micro-organisms that require oxygen for growth are called _____.
2. Micro-organisms that thrive in environments with high salt concentrations are called _____.
3. Micro-organisms that grow best at moderate temperatures (20–45 °C) are called _____.
4. Substances that inhibit bacterial growth without killing them are called _____.
5. Compounds that kill bacteria outright are called _____.
6. The essential nutrient required for nucleic acid and ATP synthesis is _____.

1.4 Culture Systems And Applications

1.4.1 Laboratory culture techniques

In microbiology, **culture systems** are methods used to grow and maintain micro-organisms under controlled conditions. Laboratory culture techniques are essential for studying bacterial physiology, identifying species, and testing their responses to different environments. The most common technique is the **agar plate culture**, where bacteria are grown on a solid medium to form visible colonies. Liquid cultures, such as **broth cultures**, allow for the study of growth dynamics and metabolic activity.

Selective and differential media are often used to isolate specific bacteria. **Selective media** contain compounds that inhibit unwanted organisms while allowing the target bacteria to grow. **Differential media** highlight differences between bacterial species, often through colour changes or biochemical reactions.

Fill in the blank: A culture medium that contains compounds to inhibit unwanted organisms while supporting the growth of target bacteria is called _____ medium.

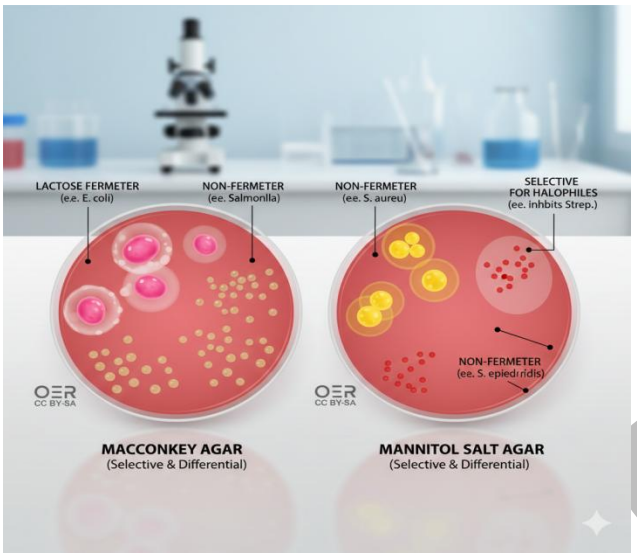

Figure 1.5 Laboratory culture techniques using agar plates

1.4.2 Industrial and research applications

Beyond the laboratory, culture systems have wide-ranging applications in industry and research. In industrial microbiology, bacteria are cultured to produce valuable products such as enzymes, antibiotics, organic acids, and biofuels. Continuous culture systems, such as chemostats, are particularly useful for large-scale production because they maintain bacteria in the exponential growth phase.

In research, culture systems are used to study bacterial genetics, physiology, and interactions with hosts. They also play a role in biotechnology, where genetically engineered bacteria are cultured to produce recombinant proteins, vaccines, and other therapeutic products. The ability to manipulate and maintain bacterial cultures under controlled conditions is therefore central to both scientific discovery and industrial innovation.

Fill in the blank: The culture system most commonly used in industry to maintain bacteria in the exponential growth phase is the _____.

Industry	Primary Applications & Key Products
Pharmaceuticals & Medicine	• **Antibiotics:** Production of penicillin (*Penicillium*), streptomycin (*Streptomyces*), and tetracycline. • **Biologics:** Recombinant proteins like insulin and human growth hormone. • **Vaccines:** Culturing bacteria for whole-cell or subunit vaccine development. • **Diagnostics:** Identifying pathogens and testing for antibiotic susceptibility (AST).

Industry	Primary Applications & Key Products
Food & Beverage	• **Fermentation:** Production of yogurt (*Lactobacillus*), cheese, and vinegar (*Acetobacter*). • **Additives:** Synthesis of amino acids (Glutamate), vitamins (B12), and organic acids (Citric acid). • **Probiotics:** Production of beneficial live cultures for gut health products.
Environmental Management	• **Bioremediation:** Using specialized cultures to break down oil spills, heavy metals, and toxic waste. • **Wastewater Treatment:** Aerobic and anaerobic digestion of organic matter in sewage and industrial runoff. • **Biocontrol:** Developing microbial pesticides (e.g., *Bacillus thuringiensis*).
Energy & Biofuels	• **Bioethanol & Methane:** Fermenting agricultural waste and biomass into renewable fuels. • **Microbial Fuel Cells (MFCs):** Harnessing bacterial metabolism to generate electricity during waste treatment.
Agriculture	• **Biofertilizers:** Culturing nitrogen-fixing bacteria (*Rhizobium*) to enhance soil fertility naturally. • **Plant Growth Promoters:** Bacteria that produce siderophores or hormones to improve crop yield.
Advanced Materials	• **Bioplastics:** Synthesis of Polyhydroxyalkanoates (PHAs) as biodegradable alternatives to petroleum plastics. • **Industrial Enzymes:** Production of proteases and amylases for detergents, textiles, and paper manufacturing.

Box 1.2 Selected industrial applications of bacterial culture systems

Pilot 1.4

1. The method used to isolate individual bacterial colonies on solid media is called the _____ technique.

2. The process of transferring microbes from one medium to another under sterile conditions is called _____.
3. The bacterium used in the production of lactic acid for food fermentation is _____.
4. The microbial process used in the production of antibiotics is called _____.
5. The large-scale cultivation of bacteria for biotechnology is referred to as _____.

Series Summary

This Series has introduced you to the fundamental aspects of bacterial anatomy, growth dynamics, and culture systems, which form the basis for understanding microbial physiology and metabolism. We began by examining the structural organisation and cytochemical composition of bacterial cells, then explored how populations grow in batch and continuous culture systems, supported by mathematical modelling. Following that, we considered the physical and chemical factors that influence bacterial growth, and finally we studied laboratory techniques and industrial applications of culture systems.

By working through these Parts, you should now be able to define bacterial structures and their functions, analyse growth phases and interpret growth curves, evaluate continuous culture systems, assess the impact of physical and chemical factors on bacterial growth, and apply knowledge of culture systems to both laboratory and industrial contexts. These abilities directly align with the Series Learning Outcomes, ensuring that you have gained the essential skills and knowledge to progress confidently in microbial physiology and metabolism.

Glossary of Terms

- **Acidophiles**: Bacteria that grow best in acidic environments with low pH values.
- **Aerobes**: Bacteria that require oxygen for growth and metabolism.
- **Agar plate culture**: A laboratory technique where bacteria are grown on a solid medium to form visible colonies.
- **Anaerobes**: Bacteria that grow in environments without oxygen.
- **Capsule**: A polysaccharide layer surrounding some bacterial cells that provides protection against host defences.
- **Chemostat**: A continuous culture system that maintains bacterial growth by regulating nutrient flow and waste removal.
- **Cytoplasm**: The gel-like substance inside bacterial cells that contains enzymes, nutrients, and ribosomes.
- **Differential media**: Culture media that distinguish between bacterial species based on biochemical reactions, often producing colour changes.
- **Exponential growth equation**: A mathematical model used to describe bacterial population increase over time, expressed as (N_t = N_0 \cdot e^{\mu t}).
- **Facultative anaerobes**: Bacteria that can grow with or without oxygen, adapting to both conditions.
- **Flagella**: Whip-like appendages that enable bacterial motility.
- **Halophiles**: Bacteria that thrive in environments with high salt concentrations.
- **Lag phase**: The initial phase of bacterial growth where cells adapt to their environment before dividing.

- **Log phase**: The phase of rapid, exponential bacterial growth.
- **Mesophiles**: Bacteria that grow best at moderate temperatures, typically between 20°C and 45°C.
- **Nucleoid**: The region in bacterial cells containing the genetic material, usually a single circular DNA molecule.
- **Peptidoglycan**: A polymer of sugars and amino acids that forms the bacterial cell wall, providing shape and protection.
- **Plasmids**: Small, circular DNA molecules in bacteria that carry additional genes, often related to antibiotic resistance.
- **Psychrophiles**: Bacteria that thrive at low temperatures, usually below 15°C.
- **Ribosomes**: Structures within the cytoplasm that serve as sites of protein synthesis.
- **Selective media**: Culture media that contain compounds to inhibit unwanted organisms while supporting the growth of target bacteria.
- **Stationary phase**: The phase of bacterial growth where nutrient depletion and waste accumulation slow down cell division.
- **Thermophiles**: Bacteria that grow best at high temperatures, often above 55°C.

Concept Check Questions [Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems]

Objective questions

1. Which bacterial structure provides shape and protection? (Linked to SLO 1.1)
   a) Ribosome
   b) Cell wall
   c) Capsule
   d) Nucleoid
2. In which phase of batch culture growth do bacteria divide at their maximum rate? (Linked to SLO 1.3)
   a) Lag phase
   b) Log phase
   c) Stationary phase
   d) Death phase
3. What is the name of the continuous culture system that maintains bacteria in the exponential phase? (Linked to SLO 1.4)
   a) Batch reactor
   b) Chemostat
   c) Fermentor
   d) Bioreactor
4. Which type of bacteria thrive in high-salt environments? (Linked to SLO 1.6)
   a) Acidophiles
   b) Halophiles
   c) Thermophiles
   d) Psychrophiles
5. A medium that inhibits unwanted organisms but supports target bacteria is called what? (Linked to SLO 1.8)
   a) Differential medium

b) Selective medium
c) Nutrient medium
d) Enrichment medium

Short-answer questions

1. Define plasmids and explain their role in bacterial physiology. (Linked to SLO 1.1)
2. Describe the difference between selective and differential media. (Linked to SLO 1.8)
3. Explain how pH influences bacterial growth, giving one example of bacteria adapted to acidic conditions. (Linked to SLO 1.6)
4. State the exponential growth equation and identify the meaning of each parameter. (Linked to SLO 1.5)
5. What is the main advantage of continuous culture systems over batch cultures? (Linked to SLO 1.4)

Long-answer questions

1. Analyse the structural organisation of bacterial cells, highlighting the functions of the cell wall, nucleoid, ribosomes, and capsule. (Linked to SLO 1.1, SLO 1.2)
2. Evaluate the impact of physical and chemical factors on bacterial growth, using examples to illustrate how these conditions influence survival and adaptation. (Linked to SLO 1.6, SLO 1.7)
3. Apply your knowledge of culture systems to discuss their importance in industrial microbiology, with reference to at least two products derived from bacterial cultures. (Linked to SLO 1.9)

Answers to Concept Check Questions [Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems]

Objective questions

1. b) Cell wall
2. b) Log phase
3. b) Chemostat
4. b) Halophiles
5. b) Selective medium

Short-answer questions

1. Plasmids are small, circular DNA molecules found in bacteria. They often carry genes that provide advantages such as antibiotic resistance.
2. Selective media inhibit unwanted organisms while supporting target bacteria, whereas differential media distinguish between species based on biochemical reactions.
3. pH affects enzyme activity and membrane stability. Acidophiles, for example, grow optimally in acidic environments.

4. $(N_t = N_0 \cdot e^{\mu t})$, where (N_t) is population at time (t), (N_0) is initial population, (μ) is specific growth rate, and (e) is the base of natural logarithms.
5. Continuous culture systems maintain bacteria in the exponential phase, allowing sustained growth and product formation.

Long-answer questions

1. ***Basic response*:** The cell wall provides shape and protection, the nucleoid contains genetic material, ribosomes synthesise proteins, and the capsule protects against host defences.
   ***Value-added response*:** In addition, the capsule enhances pathogenicity, ribosomes enable rapid protein production, and the nucleoid's organisation supports efficient replication and transcription.
2. ***Basic response*:** Physical factors such as temperature and pH determine growth ranges, while chemical factors like nutrients and toxins influence metabolism and survival.
   ***Value-added response*:** For example, thermophiles adapt to high temperatures by producing heat-stable enzymes, while bacteria exposed to antibiotics may develop resistance through plasmid-encoded genes.
3. ***Basic response*:** Culture systems are important in producing antibiotics and enzymes. Continuous cultures allow sustained production.
   ***Value-added response*:** Beyond antibiotics and enzymes, bacterial cultures are used to produce organic acids, biofuels, and recombinant proteins, demonstrating their broad industrial significance.

Answers to Pilot Questions [Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems]

Part 1: Bacterial Anatomy and Cytochemistry

1. Cytoplasm
2. Flagellum
3. Capsule
4. DNA
5. Peptidoglycan
6. ATP

Part 2: Growth Dynamics of Bacterial Cultures

1. Lag phase
2. Log (exponential) phase
3. Stationary phase
4. Continuous culture
5. Chemostat
6. Monod equation
7. μmax (maximum specific growth rate)

Part 3: Factors Affecting Bacterial Growth

1. Obligate aerobes
2. Halophiles
3. Mesophiles
4. Bacteriostatic agents
5. Bactericidal agents
6. Phosphorus

Part 4: Culture Systems and Applications

1. Streak plate technique
2. Subculturing
3. Lactobacillus
4. Fermentation
5. Bioreactor cultivation

References and Attributions [Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems]

Figures

- Figure 1.1 Structural organisation of a bacterial cell. Suggested AI prompt: "Generate a labelled diagram of a bacterial cell showing cell wall, membrane, nucleoid, ribosomes, flagella, pili, and capsule." Search string: "open educational resource bacterial cell diagram labelled."
- Figure 1.2 Bacterial growth curve in batch culture. Suggested AI prompt: "Generate a labelled bacterial growth curve showing lag, log, stationary, and death phases." Search string: "OER bacterial growth curve diagram."
- Figure 1.3 Chemostat system for continuous culture. Suggested AI prompt: "Generate a labelled diagram of a chemostat showing inflow of nutrients and outflow of culture." Search string: "OER chemostat diagram continuous culture."
- Figure 1.4 Temperature ranges for bacterial growth. Suggested AI prompt: "Generate a labelled diagram showing psychrophiles, mesophiles, and thermophiles with their temperature ranges." Search string: "OER bacterial growth temperature ranges diagram."
- Figure 1.5 Laboratory culture techniques using agar plates. Suggested AI prompt: "Generate a labelled diagram showing bacterial colonies on selective and differential agar plates." Search string: "OER bacterial culture agar plate diagram."

Tables

- Table 1.1 Cytochemical components of bacterial cells and their functions. Suggested AI prompt: "Generate a table showing bacterial cell components and their functions." Search string: "OER bacterial cell components functions table."

- Table 1.2 Essential chemical factors influencing bacterial growth. Suggested AI prompt: “Generate a table showing essential nutrients and inhibitors affecting bacterial growth.” Search string: “OER bacterial growth nutrients inhibitors table.”

Boxes

- Box 1.1 Key equations in bacterial growth modelling. Suggested AI prompt: “Generate a box summarising bacterial growth equations and parameter definitions.” Search string: “OER bacterial growth exponential equation.”
- Box 1.2 Selected industrial applications of bacterial culture systems. Suggested AI prompt: “Generate a box summarising industrial applications of bacterial culture systems.” Search string: “OER industrial applications bacterial culture systems.”

Textual references

- All definitions and explanations of bacterial structures, growth phases, and culture systems were derived from the instructional content developed for Series 1.
- Mathematical modelling of bacterial growth referenced the standard exponential growth equation widely used in microbiology.

Course Code: MCB 424
Course Title: Microbial Physiology & Metabolism
Course Unit: 3 Units

Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems
Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors
Series 3: Biochemistry of Microbial Processes and Biosynthesis
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Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors

Series Outline

2.1 Microbial Nutrition

- 2.1.1 Nutrient requirements of micro-organisms
- 2.1.2 Sources of nutrients and uptake mechanisms
- 2.1.3 Growth media and nutrient formulations

2.2 Energy Metabolism in Microbes

- 2.2.1 Catabolic pathways: glycolysis and fermentation
- 2.2.2 Aerobic respiration and the electron transport chain
- 2.2.3 Anaerobic respiration and alternative electron acceptors
- 2.2.4 ATP generation and energy yield comparisons

2.3 Environmental Factors Influencing Microbial Activity

- 2.3.1 Temperature, pH, and osmotic pressure
- 2.3.2 Oxygen availability and redox conditions
- 2.3.3 Radiation, pressure, and other stress factors

2.4 Adaptations and Ecological Roles

- 2.4.1 Microbial survival strategies under stress
- 2.4.2 Symbiotic and competitive interactions in ecosystems
- 2.4.3 Applications in biotechnology and environmental microbiology

Introduction

In the last Series, we explored bacterial anatomy, growth dynamics, and culture systems, laying the foundation for understanding how micro-organisms are structured and how they behave under different conditions. Building on that knowledge, we now turn to the ways in which microbes obtain nutrients, generate energy, and adapt to their environments. These processes are central to microbial survival and productivity, and they underpin applications in medicine, biotechnology, and environmental science.

The aim of this Series is to equip you with the ability to explain microbial nutritional requirements, analyse energy metabolism pathways, evaluate the influence of environmental factors, and apply this knowledge to microbial adaptations and ecological roles.

We shall commence this Series by examining microbial nutrition, focusing on nutrient requirements, sources, and uptake mechanisms, as well as the design of growth media. Next, we will explore energy metabolism in microbes, beginning with catabolic pathways such as glycolysis and fermentation, then moving on to aerobic and anaerobic respiration, and concluding with ATP generation and energy yield comparisons. Following that, we will consider environmental factors influencing microbial activity, including temperature, pH, osmotic pressure, oxygen availability, and other stress conditions. Finally, we will wrap up by studying microbial adaptations and ecological roles, highlighting survival strategies, symbiotic and competitive interactions, and applications in biotechnology and environmental microbiology. This progression will help you connect nutritional and metabolic processes with environmental influences and practical applications.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **2.1** define the nutrient requirements of micro-organisms and explain how they obtain and utilise these nutrients,

- **2.2** describe sources of nutrients and demonstrate how uptake mechanisms support microbial growth,
- **2.3** analyse the composition of growth media and evaluate their suitability for different microbial species,
- **2.4** explain catabolic pathways such as glycolysis and fermentation, highlighting their role in microbial energy metabolism,
- **2.5** evaluate aerobic respiration by outlining the electron transport chain and comparing energy yields,
- **2.6** examine anaerobic respiration and assess the use of alternative electron acceptors,
- **2.7** calculate ATP yield across different metabolic pathways and compare efficiency of energy generation,
- **2.8** assess the influence of environmental factors such as temperature, pH, osmotic pressure, and oxygen availability on microbial activity,
- **2.9** evaluate microbial adaptations to stress conditions and explain their ecological significance, and
- **2.10** apply knowledge of microbial nutrition, metabolism, and environmental factors to discuss their roles in biotechnology and environmental microbiology.

2.1 Microbial Nutrition

2.1.1 Nutrient requirements of micro-organisms

Micro-organisms require a variety of **nutrients**, defined as chemical substances that provide energy and building blocks for growth and metabolism. The major categories include **macronutrients**, such as carbon, nitrogen, sulphur, phosphorus, and oxygen, which are needed in large amounts, and **micronutrients**, such as iron, magnesium, and zinc, which are required in smaller quantities but are essential for enzymatic activity.

Carbon serves as the backbone of organic molecules, nitrogen is vital for proteins and nucleic acids, sulphur contributes to amino acids and vitamins, and phosphorus is a key component of nucleotides and cell membranes. Micronutrients act as cofactors, enabling enzymes to function properly.

Fill in the blank: The nutrient that forms the backbone of organic molecules in micro-organisms is _____.

Category	Nutrient	Symbol	Primary Functions & Cellular Roles
Macronutrients	**Carbon**	C	Structural backbone of all organic molecules (lipids, proteins, etc.).
	Oxygen	O	Constituent of cell water and organic compounds; terminal electron acceptor in aerobic respiration.
	Hydrogen	H	Component of organic compounds and cell water; important in pH and redox reactions.

Category	Nutrient	Symbol	Primary Functions & Cellular Roles
	Nitrogen	N	Essential for synthesis of amino acids, proteins, nucleotides (DNA/RNA), and ATP.
	Phosphorus	P	Core component of nucleic acids, phospholipids (cell membranes), and ATP.
	Sulfur	S	Needed for sulfur-containing amino acids (cysteine, methionine) and certain vitamins (biotin, thiamine).
	Potassium	K	Vital for protein synthesis and serves as a cofactor for numerous enzymes.
	Magnesium	Mg	Stabilizes ribosomes, cell membranes, and nucleic acids; essential enzyme cofactor.
	Calcium	Ca	Contributes to the heat resistance of bacterial endospores; stabilizes cell walls.
	Iron	Fe	Key component of cytochromes and iron-sulfur proteins involved in electron transport/respiration.
Micronutrients	**Zinc**	Zn	Found in the active site of many enzymes (e.g., DNA polymerase, alcohol dehydrogenase).
(Trace Elements)	**Manganese**	Mn	Activates enzymes involved in phosphate transfer and oxygen evolution.
	Molybdenum	Mo	Required for nitrogen fixation (nitrogenase) and nitrate reduction.
	Cobalt	Co	Essential component of Vitamin B12 and its derivatives.
	Copper	Cu	Involved in respiration as a cofactor for certain terminal oxidases.
	Nickel	Ni	Cofactor for enzymes like urease and hydrogenases.

Table 2.1 Major nutrient requirements of micro-organisms

2.1.2 Sources of nutrients and uptake mechanisms

Micro-organisms obtain nutrients from diverse sources. **Autotrophs** use carbon dioxide as their carbon source, while **heterotrophs** rely on organic compounds. Similarly, **phototrophs** derive energy from light, whereas **chemotrophs** obtain energy from chemical compounds.

Nutrient uptake occurs through specialised mechanisms. **Passive diffusion** allows small molecules to move across membranes without energy expenditure. **Facilitated diffusion** uses carrier proteins to transport molecules more efficiently. **Active transport** requires energy, often in the form of ATP, to move nutrients against concentration gradients. Some bacteria also employ **group translocation**, a process where transported molecules are chemically modified during entry.

Fill in the blank: Micro-organisms that use carbon dioxide as their carbon source are called _____.

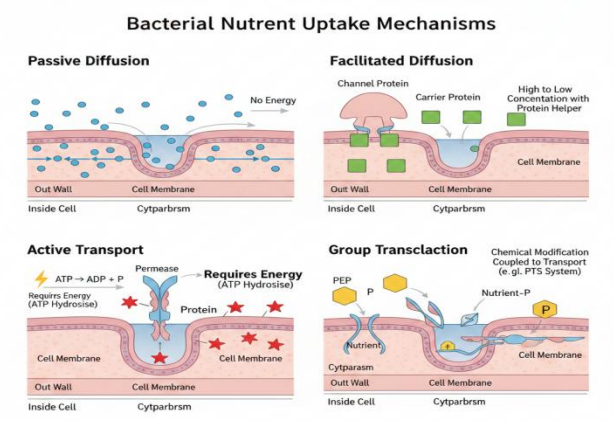


Figure 2.1 Nutrient uptake mechanisms in micro-organisms

2.1.3 Growth media and nutrient formulations

To study micro-organisms in the laboratory, scientists use **growth media**, defined as prepared nutrient solutions that support microbial growth. Media can be **synthetic (defined)**, where all chemical components are known, or **complex**, which contain extracts such as yeast or peptone with unknown exact composition.

Specialised media include **selective media**, which inhibit unwanted organisms while supporting target bacteria, and **differential media**, which distinguish between species based on biochemical reactions. Enrichment media are designed to promote the growth of specific organisms by providing favourable nutrients.

Fill in the blank: A growth medium in which all chemical components are known is called _____ medium.

Media Type	Description	Key Examples
Defined (Synthetic)	All chemical components and their exact concentrations are known.	M9 Minimal Media, *E. coli* minimal medium.

Media Type	Description	Key Examples
Complex (Non-synthetic)	Contains "extracts" (yeast, beef, soy) or digests of proteins; exact chemical composition varies.	Nutrient Broth (NB), Tryptic Soy Agar (TSA).
Selective	Contains agents that inhibit the growth of unwanted microbes while encouraging others.	Phenylethyl Alcohol (PEA) Agar (inhibits Gram-negatives).
Differential	Contains indicators (like pH dyes) that allow researchers to distinguish between different species based on their metabolism.	Blood Agar (shows hemolysis), Phenol Red Broths.
Selective & Differential	Combines both functions; inhibits certain groups while distinguishing between those that do grow.	**MacConkey Agar** (inhibits Gram-positives; differentiates lactose fermenters).
Enriched	Contains extra nutrients (blood, serum, vitamins) to support the growth of fastidious ("picky") organisms.	Chocolate Agar, Blood Agar.

Box 2.1 Types of growth media used in microbiology

Pilot 2.1

1. Which nutrient forms the backbone of organic molecules in micro-organisms?
2. Micro-organisms that use carbon dioxide as their carbon source are called _____.
3. A growth medium in which all chemical components are known is called _____ medium.

2.2 Energy Metabolism In Microbes

2.2.1 Catabolic pathways: glycolysis and fermentation

Micro-organisms break down nutrients to release energy through **catabolic pathways**, defined as metabolic routes that degrade molecules into simpler forms while producing energy. The most fundamental pathway is **glycolysis**, where glucose is converted into pyruvate, yielding ATP and NADH. This process occurs in the cytoplasm and does not require oxygen.

When oxygen is absent, many microbes switch to **fermentation**, a pathway that regenerates NAD^+ by converting pyruvate into products such as lactic acid, ethanol, or other organic compounds. Although fermentation produces less ATP than respiration, it allows continued energy generation under anaerobic conditions.

Fill in the blank: The metabolic pathway that converts glucose into pyruvate while producing ATP is called _____.

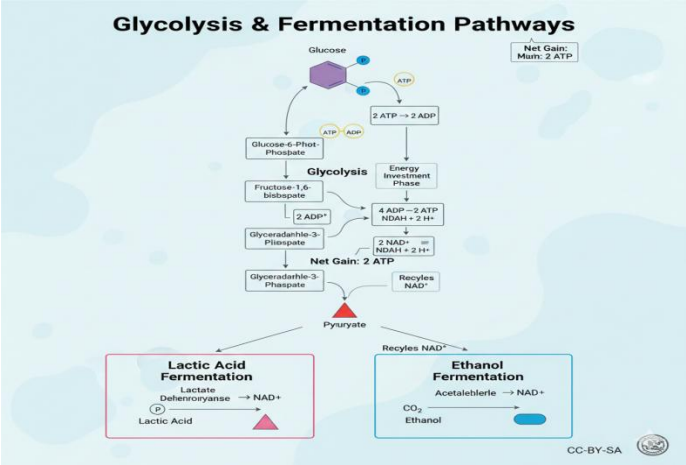


Figure 2.2 Glycolysis and fermentation in microbial metabolism

2.2.2 Aerobic respiration and the electron transport chain

In the presence of oxygen, microbes use **aerobic respiration**, defined as the complete oxidation of glucose to carbon dioxide and water, yielding high amounts of ATP. After glycolysis, pyruvate enters the tricarboxylic acid (TCA) cycle, producing NADH and $FADH_2$. These electron carriers feed into the **electron transport chain (ETC)**, a series of protein complexes embedded in the cell membrane that transfer electrons and pump protons, creating a proton gradient.

The gradient drives **ATP synthase**, an enzyme that generates ATP from ADP and inorganic phosphate. Aerobic respiration is highly efficient, producing up to 38 ATP molecules per glucose in bacteria.

Fill in the blank: The enzyme that uses a proton gradient to generate ATP during aerobic respiration is called _____.

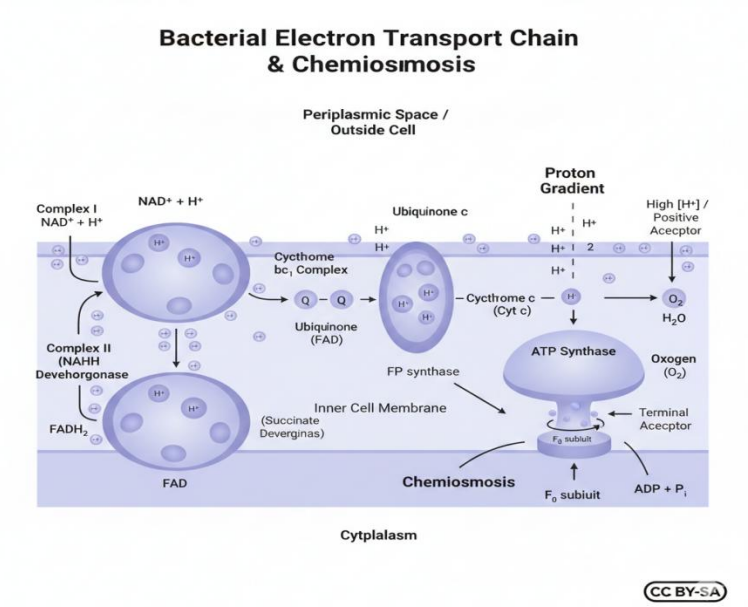


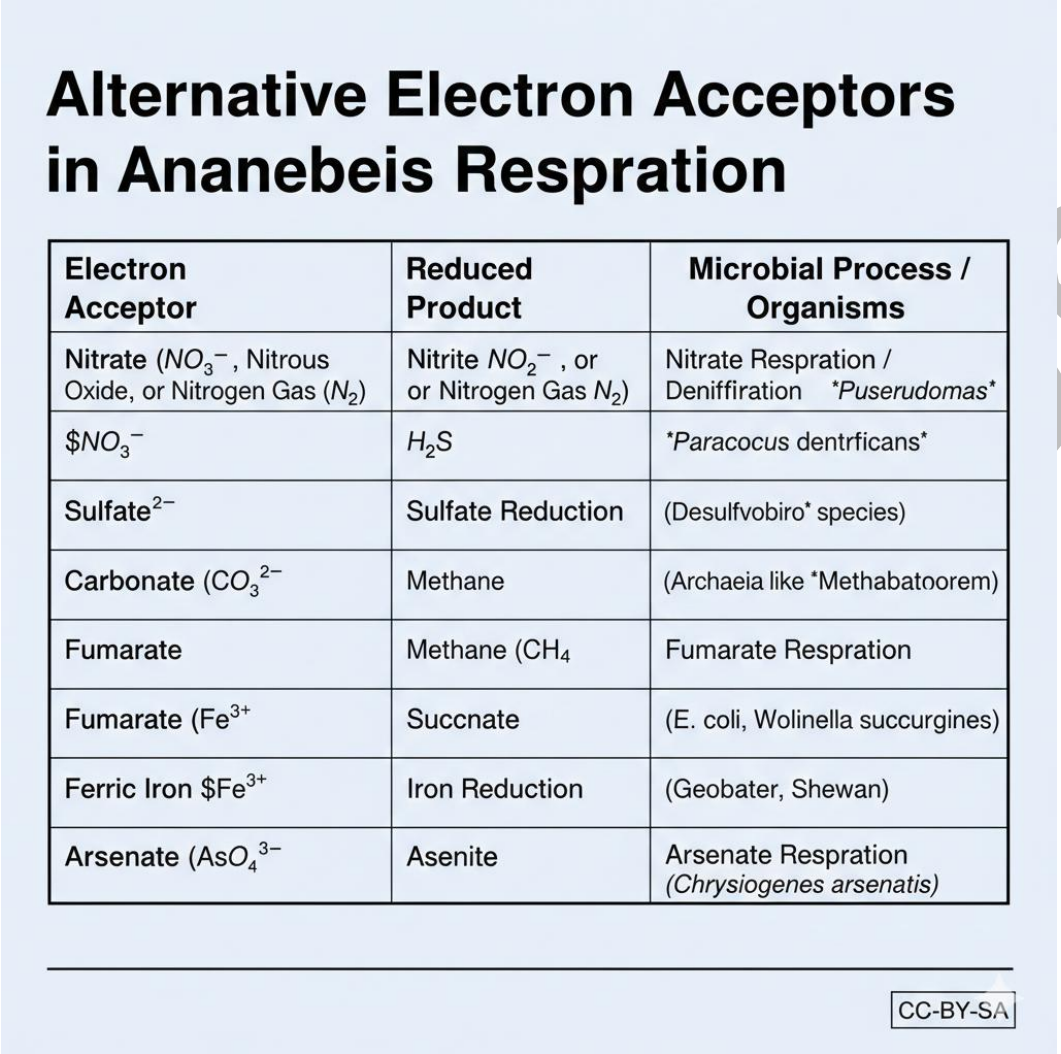
Figure 2.3 Aerobic respiration and the electron transport chain

2.2.3 Anaerobic respiration and alternative electron acceptors

Some microbes perform **anaerobic respiration**, defined as energy generation using electron transport chains without oxygen. Instead of oxygen, they use alternative **electron acceptors** such as nitrate, sulphate, or carbon dioxide. This process allows microbes to survive in oxygen-limited environments while still producing ATP through oxidative phosphorylation.

Anaerobic respiration is less efficient than aerobic respiration, but it is crucial in ecological processes such as nitrogen cycling and methane production.

Fill in the blank: In anaerobic respiration, microbes may use _____ as an alternative electron acceptor instead of oxygen.



Alternative Electron Acceptors in Ananebeis Respration

Electron Acceptor	Reduced Product	Microbial Process / Organisms
Nitrate (NO_3^-, Nitrous Oxide, or Nitrogen Gas (N_2)	Nitrite NO_2^-, or or Nitrogen Gas N_2)	Nitrate Respration / Deniffiration **Puserudomas**
$\$NO_3^-$	H_2S	**Paracocus* dentrficans*
Sulfate^{2-}	Sulfate Reduction	(Desulfvobiro* species)
Carbonate (CO_3^{2-}	Methane	(Archaeia like *Methabatoorem)
Fumarate	Methane (CH_4	Fumarate Respration
Fumarate (Fe^{3+}	Succnate	(E. coli, Wolinella succurgines)
Ferric Iron $\$Fe^{3+}$	Iron Reduction	(Geobater, Shewan)
Arsenate (AsO_4^{3-}	Asenite	Arsenate Respration *(Chrysiogenes arsenatis)*

CC-BY-SA

Table 2.2 Alternative electron acceptors used in anaerobic respiration

2.2.4 ATP generation and energy yield comparisons

The efficiency of microbial metabolism can be compared by examining **ATP yield**, defined as the number of ATP molecules produced per glucose molecule. Glycolysis alone yields 2 ATP, fermentation also yields 2 ATP, anaerobic respiration yields variable amounts depending on the electron acceptor, and aerobic respiration yields the highest, up to 38 ATP.

These differences explain why microbes adapt their metabolism to environmental conditions. In oxygen-rich environments, aerobic respiration is favoured, while in oxygen-poor conditions, fermentation or anaerobic respiration ensures survival.

Fill in the blank: The metabolic process that yields the highest number of ATP molecules per glucose is _____.

Metabolic Process	Terminal Electron Acceptor	Energy Investment	Final ATP Yield (per Glucose)	Efficiency
Glycolysis (alone)	Internal (NAD+ recycling needed)	2 ATP	**2 ATP** (Net)	Low
Fermentation	Organic molecule (e.g., Pyruvate)	2 ATP	**2 ATP** (Net)	Low
Anaerobic Respiration	Inorganic molecule (e.g., NO_3^-, SO_4^{2-})	2 ATP	**Variable (2 – 30+ ATP)**	Moderate
Aerobic Respiration	Oxygen (O_2)	2 ATP	**32 – 38 ATP**	High

Box 2.2 ATP yield in microbial metabolic pathways

Pilot 2.2

1. The metabolic pathway that converts glucose into pyruvate while producing ATP is called _____.
2. The enzyme that uses a proton gradient to generate ATP during aerobic respiration is called _____.
3. In anaerobic respiration, microbes may use _____ as an alternative electron acceptor instead of oxygen.
4. The metabolic process that yields the highest number of ATP molecules per glucose is _____.

2.3 Environmental Factors Influencing Microbial Activity

2.3.1 Temperature, pH, and osmotic pressure

Microbial activity is strongly influenced by **temperature**, defined as the degree of heat present in the environment. Different groups of microbes thrive at different ranges: **psychrophiles** prefer cold conditions, **mesophiles** grow best at moderate temperatures, and **thermophiles** flourish in high heat.

Another critical factor is **pH**, which refers to the acidity or alkalinity of the environment. **Acidophiles** grow in acidic conditions, while **alkaliphiles** prefer alkaline environments. Most bacteria are **neutrophiles**, thriving near neutral pH.

Osmotic pressure, the force exerted by solute concentrations across membranes, also affects microbial survival. **Halophiles** are adapted to high salt concentrations, while most bacteria require balanced osmotic conditions to prevent cell damage.

Fill in the blank: Micro-organisms that thrive in environments with high salt concentrations are called _____.

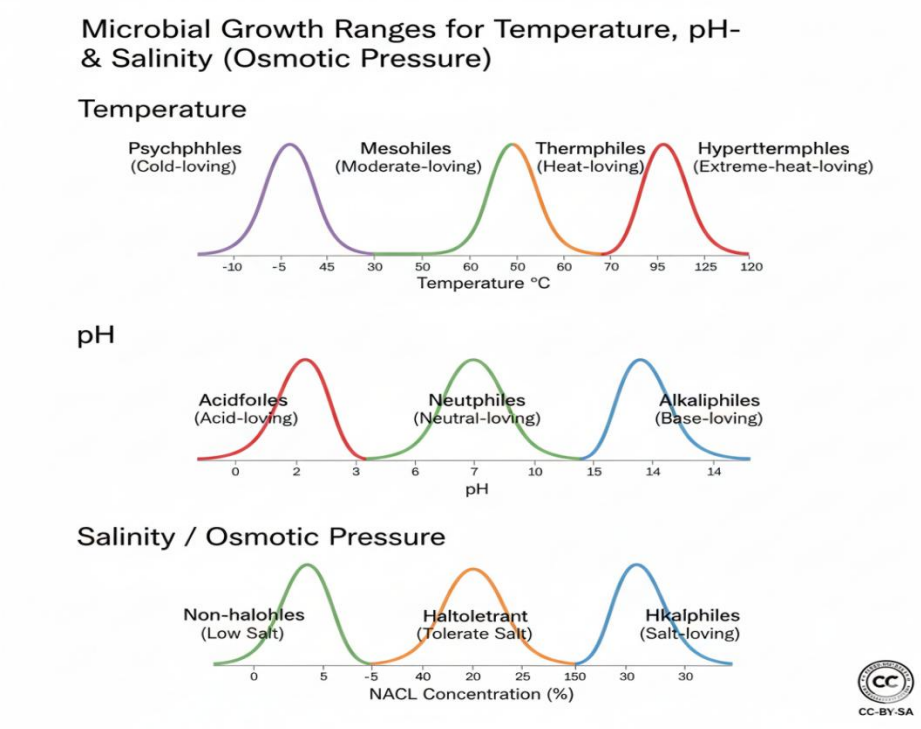


Figure 2.4 Environmental factors influencing microbial growth

2.3.2 Oxygen availability and redox conditions

Microbes differ in their requirements for **oxygen**, defined as the gaseous element essential for aerobic respiration. **Obligate aerobes** require oxygen, **obligate anaerobes** cannot tolerate it, and **facultative anaerobes** can grow with or without oxygen. **Microaerophiles** need small amounts of oxygen, while **aerotolerant anaerobes** do not use oxygen but can survive in its presence. Environmental **redox conditions**, which describe the balance between oxidising and reducing agents, also influence microbial metabolism. Some microbes thrive in highly reduced environments, such as sediments, while others require oxidising conditions.
Fill in the blank: Micro-organisms that require oxygen for growth are called _____.

Group	Oxygen Requirement	Growth Pattern in Tube	Presence of Enzymes (SOD/Catalase)
Obligate Aerobes	Required for growth.	Only at the very top (high O_2 concentration).	**Present:** Both SOD and Catalase.
Obligate Anaerobes	Toxic; growth ceases in its presence.	Only at the bottom (no O_2).	**Absent:** Lacks protective enzymes.

Group	Oxygen Requirement	Growth Pattern in Tube	Presence of Enzymes (SOD/Catalase)
Facultative Anaerobes	Greater growth with O_2, but can grow without it.	Best at top, but diffused throughout the tube.	**Present:** Both SOD and Catalase.
Aerotolerant Anaerobes	Does not use O_2, but tolerates its presence.	Even growth throughout the entire tube.	**Present:** SOD; (usually lacks Catalase).
Microaerophiles	Required in low concentrations (2–10%).	A thin band just below the surface.	**Present:** Low levels of enzymes.

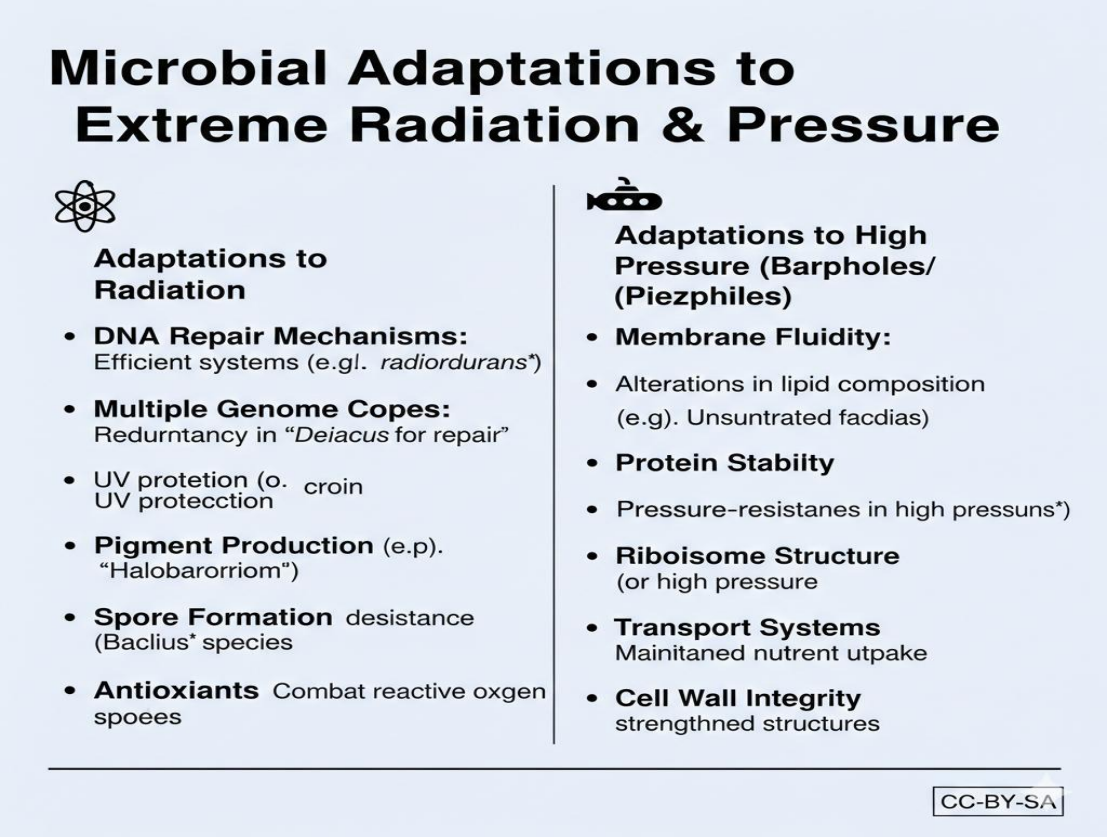
Table 2.3 Microbial groups based on oxygen requirements

2.3.3 Radiation, pressure, and other stress factors

Microbes are also influenced by **radiation**, defined as energy emitted in the form of waves or particles. While high levels of ultraviolet (UV) radiation can damage DNA, some microbes such as **radiophiles** have mechanisms to repair radiation-induced damage.

Pressure is another environmental factor. **Barophiles** or **piezophiles** thrive under high hydrostatic pressure, such as in deep-sea environments. Other stress factors include desiccation (drying), toxic chemicals, and heavy metals, all of which can limit microbial activity unless specific adaptations are present.

Fill in the blank: Micro-organisms that thrive under high hydrostatic pressure are called _____.



Box 2.3

Microbial adaptations to radiation and pressure

Pilot 2.3

1. Micro-organisms that thrive in environments with high salt concentrations are called _____.
2. Micro-organisms that require oxygen for growth are called _____.
3. Micro-organisms that thrive under high hydrostatic pressure are called _____.

2.4 Adaptations And Ecological Roles

2.4.1 Microbial survival strategies under stress

Micro-organisms have evolved remarkable **adaptations**, defined as structural or functional changes that enable survival under challenging conditions. For example, some bacteria form **endospores**, which are highly resistant dormant structures that protect genetic material during extreme heat, desiccation, or radiation. Others produce protective pigments or enzymes that neutralise harmful substances.

These strategies ensure that microbes can persist in diverse environments, from soil and water to the human body. By adopting survival mechanisms, they maintain ecological balance and continue to contribute to nutrient cycling.

Fill in the blank: The resistant dormant structures formed by some bacteria to survive extreme conditions are called _____.

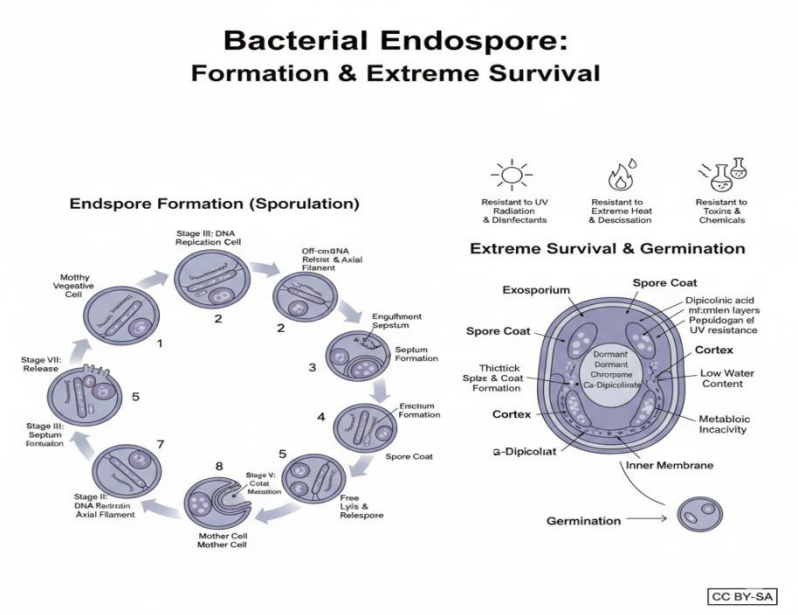


Figure 2.5 Microbial survival strategies under stress

2.4.2 Symbiotic and competitive interactions in ecosystems

Microbes play vital roles in ecosystems through **symbiosis**, defined as close and long-term interactions between different species. Examples include **mutualism**, where both partners benefit (such as nitrogen-fixing bacteria in plant roots), and **commensalism**, where one benefits without harming the other. In contrast, **parasitism** involves one organism benefiting at the expense of the host.

Microbes also engage in **competition**, where they vie for nutrients and space. Some produce **antibiotics**, chemical compounds that inhibit competitors, giving them an ecological advantage. These interactions shape microbial communities and influence biodiversity.

Fill in the blank: The ecological interaction where both partners benefit is called _____.

Interaction Type	Effect on Organism A	Effect on Organism B	Description	Example
Mutualism	Beneficial (+)	Beneficial (+)	An obligatory relationship where both partners depend on each other for survival.	**Lichens:** A mutualism between a fungus and an alga/cyanobacterium.
Cooperation	Beneficial (+)	Beneficial (+)	A non-obligatory relationship where both benefit but can live apart.	*Azotobacter* and *Cellulomonas* working together in soil.
Commensalism	Beneficial (+)	Neutral (0)	One organism benefits while the other is neither helped nor harmed.	*Staphylococcus epidermidis* living on human skin.
Parasitism	Beneficial (+)	Harmful (-)	One organism (parasite) benefits at the expense of the host.	*Bdellovibrio* bacteria preying on Gram-negative bacteria.
Amensalism	Neutral (0)	Harmful (-)	One organism produces a substance that inhibits the growth of another.	**Antibiosis:** *Penicillium* fungi producing penicillin to kill bacteria.
Competition	Harmful (-)	Harmful (-)	Two organisms vie for the same limited	Different bacterial species competing for

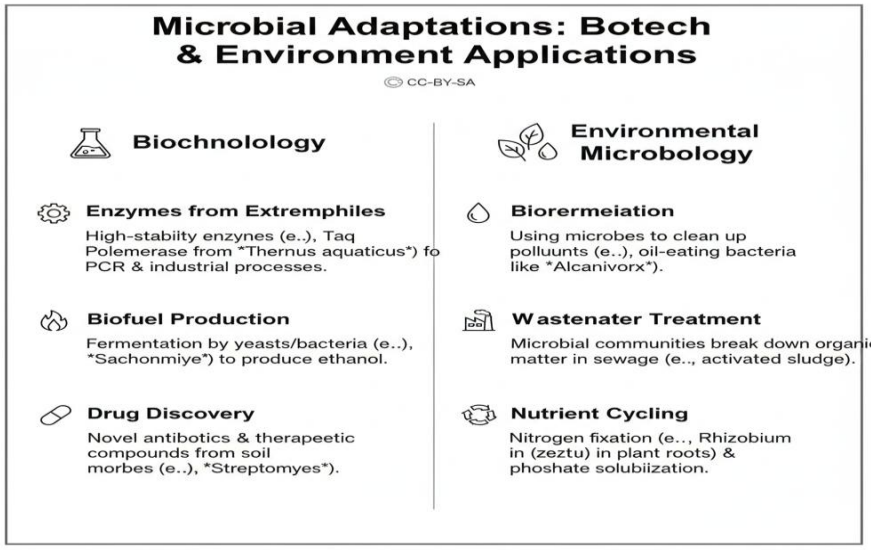
Interaction Type	Effect on Organism A	Effect on Organism B	Description	Example
			resource (nutrients, space).	colonization sites in the gut.

Table 2.4 Types of microbial interactions in ecosystems

2.4.3 Applications in biotechnology and environmental microbiology

Microbial adaptations and ecological roles have significant applications. In **biotechnology**, microbes are harnessed to produce antibiotics, enzymes, biofuels, and recombinant proteins. Their ability to adapt to stress makes them useful in industrial processes that require resilience. In **environmental microbiology**, microbes contribute to bioremediation, defined as the use of living organisms to clean up pollutants such as oil spills or heavy metals. They also play roles in waste treatment, nutrient recycling, and maintaining soil fertility. These applications highlight the importance of microbial ecology in solving global challenges.

Fill in the blank: The process of using living organisms to clean up pollutants in the environment is called _____.



Box 2.4 Applications of microbial adaptations in biotechnology and environmental microbiology

Pilot 2.4

1. The resistant dormant structures formed by some bacteria to survive extreme conditions are called _____.
2. The ecological interaction where both partners benefit is called _____.
3. The process of using living organisms to clean up pollutants in the environment is called _____.

Series Summary

This Series has guided you through the essential aspects of microbial nutrition, energy metabolism, and environmental factors, all of which are central to understanding how micro-organisms survive, grow, and interact with their surroundings. We began by examining nutrient requirements, sources, and uptake mechanisms, followed by the study of growth media and their formulations. Then we explored energy metabolism, covering glycolysis, fermentation, aerobic and anaerobic respiration, and ATP yield comparisons. Moving on, we considered environmental influences such as temperature, pH, osmotic pressure, oxygen availability, radiation, and pressure. Finally, we concluded with microbial adaptations and ecological roles, highlighting survival strategies, symbiotic and competitive interactions, and applications in biotechnology and environmental microbiology.

By completing this Series, you should now be able to define microbial nutrient needs, explain uptake mechanisms, analyse growth media, describe and evaluate metabolic pathways, assess environmental influences, and apply knowledge of microbial adaptations to practical contexts. These abilities directly align with the Series Learning Outcomes, ensuring that you can confidently explain how nutrition, metabolism, and environmental factors shape microbial life and its applications in science and industry.

Glossary of Terms

- **Active transport**: A nutrient uptake mechanism where cells use energy, often in the form of ATP, to move molecules against their concentration gradient.
- **Aerobic respiration**: A metabolic process in which glucose is fully oxidised to carbon dioxide and water using oxygen, producing large amounts of ATP.
- **Anaerobic respiration**: A metabolic process that uses alternative electron acceptors such as nitrate or sulphate instead of oxygen to generate energy.
- **Antibiotics**: Chemical compounds produced by some microbes to inhibit or kill competing organisms.
- **ATP synthase**: An enzyme that generates ATP by using the proton gradient created during respiration.
- **Autotrophs**: Micro-organisms that use carbon dioxide as their carbon source.
- **Bioremediation**: The process of using living organisms, often microbes, to clean up pollutants in the environment.
- **Catabolic pathways**: Metabolic routes that break down molecules into simpler forms while releasing energy.
- **Commensalism**: A type of symbiotic relationship where one organism benefits while the other is neither harmed nor helped.
- **Endospores**: Resistant dormant structures formed by some bacteria to survive extreme conditions such as heat or desiccation.
- **Facilitated diffusion**: A nutrient uptake mechanism where carrier proteins help transport molecules across membranes without energy expenditure.
- **Fermentation**: An anaerobic metabolic process that converts pyruvate into products such as lactic acid or ethanol, regenerating NAD^+ and producing small amounts of ATP.
- **Growth media**: Prepared nutrient solutions used in laboratories to support microbial growth.
- **Halophiles**: Micro-organisms that thrive in environments with high salt concentrations.

- **Mesophiles**: Micro-organisms that grow best at moderate temperatures, typically between 20°C and 45°C.
- **Mutualism**: A type of symbiotic relationship where both organisms benefit.
- **Osmotic pressure**: The force exerted by solute concentrations across membranes, affecting microbial survival.
- **Passive diffusion**: A nutrient uptake mechanism where molecules move across membranes without energy input, following concentration gradients.
- **Phototrophs**: Micro-organisms that derive energy from light.
- **Psychrophiles**: Micro-organisms that thrive at low temperatures, usually below 15°C.
- **Radiophiles**: Micro-organisms adapted to survive high levels of radiation.
- **Symbiosis**: Close and long-term interactions between different species, which may be mutualistic, commensal, or parasitic.
- **Thermophiles**: Micro-organisms that grow best at high temperatures, often above 55°C.

Concept Check Questions [Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors]

Objective questions

1. Which nutrient serves as the backbone of organic molecules in micro-organisms? (Linked to SLO 2.1)
   a) Nitrogen
   b) Carbon
   c) Sulphur
   d) Phosphorus
2. Micro-organisms that use carbon dioxide as their carbon source are called: (Linked to SLO 2.2)
   a) Heterotrophs
   b) Autotrophs
   c) Chemotrophs
   d) Phototrophs
3. Which metabolic pathway converts glucose into pyruvate while producing ATP? (Linked to SLO 2.4)
   a) Fermentation
   b) Glycolysis
   c) Electron transport chain
   d) Anaerobic respiration
4. The enzyme that generates ATP using a proton gradient is: (Linked to SLO 2.5)
   a) DNA polymerase
   b) ATP synthase
   c) RNA polymerase
   d) Lactase
5. Micro-organisms that thrive under high hydrostatic pressure are called: (Linked to SLO 2.9)
   a) Halophiles
   b) Psychrophiles

c) Barophiles
d) Radiophiles

Short-answer questions

1. Define growth media and explain the difference between synthetic and complex media. (Linked to SLO 2.3)
2. Describe how facultative anaerobes differ from obligate anaerobes. (Linked to SLO 2.8)
3. State the ATP yield from glycolysis alone and compare it with aerobic respiration. (Linked to SLO 2.7)
4. Explain the ecological role of mutualistic microbial interactions, giving one example. (Linked to SLO 2.9)
5. What is bioremediation and why is it important in environmental microbiology? (Linked to SLO 2.10)

Long-answer questions

1. Analyse microbial nutrient requirements, distinguishing between macronutrients and micronutrients, and explain their roles in physiology. (Linked to SLO 2.1, SLO 2.2)
2. Evaluate the differences between aerobic and anaerobic respiration, including electron acceptors and ATP yield. (Linked to SLO 2.5, SLO 2.6, SLO 2.7)
3. Apply your knowledge of microbial adaptations to discuss their survival strategies under stress and their ecological significance. (Linked to SLO 2.9)
4. Assess the applications of microbial metabolism and ecological roles in biotechnology and environmental microbiology. (Linked to SLO 2.10)

Answers to Concept Check Questions [Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors]

Objective questions

1. b) Carbon
2. b) Autotrophs
3. b) Glycolysis
4. b) ATP synthase
5. c) Barophiles

Short-answer questions

1. Growth media are prepared nutrient solutions that support microbial growth. Synthetic media have known chemical compositions, while complex media contain extracts with unknown exact composition.
2. Facultative anaerobes can grow with or without oxygen, while obligate anaerobes cannot tolerate oxygen.
3. Glycolysis yields 2 ATP per glucose, while aerobic respiration yields up to 38 ATP per glucose.

4. Mutualistic interactions benefit both partners, for example nitrogen-fixing bacteria in plant roots provide nitrogen while receiving carbohydrates.
5. Bioremediation is the use of living organisms to clean up pollutants. It is important for restoring ecosystems and reducing environmental damage.

Long-answer questions

1. ***Basic response*:** Macronutrients such as carbon, nitrogen, sulphur, and phosphorus are needed in large amounts, while micronutrients like iron and magnesium are required in smaller amounts for enzyme activity.
   ***Value-added response*:** Macronutrients form the structural and functional basis of cells, while micronutrients act as cofactors in enzymatic reactions, enabling diverse metabolic processes.
2. ***Basic response*:** Aerobic respiration uses oxygen as the final electron acceptor, producing high ATP yields, while anaerobic respiration uses alternatives such as nitrate or sulphate, yielding less ATP.
   ***Value-added response*:** Aerobic respiration is more efficient, while anaerobic respiration supports ecological processes like denitrification and methanogenesis, contributing to nutrient cycling.
3. ***Basic response*:** Microbes adapt through strategies such as endospore formation, pigment production, and enzyme activity to survive stress.
   ***Value-added response*:** These adaptations allow microbes to colonise extreme environments, maintain biodiversity, and play roles in ecological resilience.
4. ***Basic response*:** Microbial metabolism and ecological roles are applied in producing antibiotics, enzymes, and biofuels, as well as in bioremediation.
   ***Value-added response*:** Beyond industrial uses, microbes contribute to waste treatment, soil fertility, and sustainable environmental management, making them vital in biotechnology and ecology.

Answers to Pilot Questions [Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors]

Part 2.1 Microbial Nutrition

- Carbon
- Autotrophs
- Synthetic (defined) medium

Part 2.2 Energy Metabolism in Microbes

- Glycolysis
- ATP synthase
- Nitrate (or other alternative electron acceptors)
- Aerobic respiration

Part 2.3 Environmental Factors Influencing Microbial Activity

- Halophiles
- Obligate aerobes
- Barophiles

Part 2.4 Adaptations and Ecological Roles

- Endospores
- Mutualism
- Bioremediation

References and Attributions [Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors]

Figures

- Figure 2.2 Glycolysis and fermentation in microbial metabolism. AI-generated using the prompt: “Generate a labelled diagram showing glycolysis steps leading to pyruvate and fermentation pathways producing lactic acid and ethanol.” Suggested OER source: “OER glycolysis fermentation diagram microbiology.”
- Figure 2.3 Aerobic respiration and the electron transport chain. AI-generated using the prompt: “Generate a labelled diagram of the bacterial electron transport chain showing NADH, $FADH_2$, proton gradient, and ATP synthase.” Suggested OER source: “OER bacterial electron transport chain diagram.”
- Figure 2.4 Environmental factors influencing microbial growth. AI-generated using the prompt: “Generate a labelled diagram showing psychrophiles, mesophiles, thermophiles, acidophiles, alkaliphiles, and halophiles with their growth ranges.” Suggested OER source: “OER microbial growth temperature pH osmotic pressure diagram.”
- Figure 2.5 Microbial survival strategies under stress. AI-generated using the prompt: “Generate a labelled diagram showing bacterial endospore formation and survival under extreme conditions.” Suggested OER source: “OER bacterial endospore survival diagram.”

Tables

- Table 2.1 Major nutrient requirements of micro-organisms. AI-generated using the prompt: “Generate a table showing macronutrients and micronutrients required by micro-organisms and their functions.” Suggested OER source: “OER microbial nutrient requirements table.”
- Table 2.2 Alternative electron acceptors used in anaerobic respiration. AI-generated using the prompt: “Generate a table showing alternative electron acceptors in anaerobic respiration and their microbial processes.” Suggested OER source: “OER anaerobic respiration electron acceptors table.”
- Table 2.3 Microbial groups based on oxygen requirements. AI-generated using the prompt: “Generate a table showing microbial groups based on oxygen requirements and

their growth characteristics." Suggested OER source: "OER microbial oxygen requirements table."
- Table 2.4 Types of microbial interactions in ecosystems. AI-generated using the prompt: "Generate a table summarising microbial interactions such as mutualism, commensalism, parasitism, and competition with examples." Suggested OER source: "OER microbial interactions ecosystems table."

Boxes

- Box 2.1 Types of growth media used in microbiology. AI-generated using the prompt: "Generate a box summarising types of growth media in microbiology with examples." Suggested OER source: "OER types of growth media microbiology."
- Box 2.2 ATP yield in microbial metabolic pathways. AI-generated using the prompt: "Generate a box comparing ATP yields from glycolysis, fermentation, anaerobic respiration, and aerobic respiration." Suggested OER source: "OER ATP yield microbial metabolism comparison."
- Box 2.3 Microbial adaptations to radiation and pressure. AI-generated using the prompt: "Generate a box summarising microbial adaptations to radiation and pressure with examples." Suggested OER source: "OER microbial adaptations radiation pressure."
- Box 2.4 Applications of microbial adaptations in biotechnology and environmental microbiology. AI-generated using the prompt: "Generate a box summarising applications of microbial adaptations in biotechnology and environmental microbiology." Suggested OER source: "OER microbial biotechnology environmental microbiology applications."

Textual references

- Explanations of microbial nutrition, energy metabolism, and environmental factors were derived from the instructional content developed for Series 2.
- Standard microbiology references on glycolysis, respiration, fermentation, and microbial ecology informed the descriptions of metabolic pathways and ecological roles.
- Definitions of key terms such as autotrophs, halophiles, endospores, and bioremediation were aligned with widely accepted microbiology textbooks and open educational resources.

Course Code: MCB 424
Course Title: Microbial Physiology & Metabolism
Course Unit: 3 Units

Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems
Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors
Series 3: Biochemistry of Microbial Processes and Biosynthesis
Series 4: Bioenergetics, Enzyme Action, and Metabolic Pathways
Series 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Series 3: Biochemistry of Microbial Processes and Biosynthesis

Series Outline

3.1 Enzymes and Catalysis in Microbial Systems

- 3.1.1 Structure and function of microbial enzymes
- 3.1.2 Mechanisms of enzyme catalysis
- 3.1.3 Regulation of enzyme activity in microbes

3.2 Metabolic Pathways and Biochemical Cycles

- 3.2.1 Carbohydrate metabolism: glycolysis, TCA cycle, and pentose phosphate pathway
- 3.2.2 Lipid metabolism and fatty acid biosynthesis
- 3.2.3 Protein metabolism and amino acid biosynthesis
- 3.2.4 Integration of metabolic pathways in microbial physiology

3.3 Biosynthesis of Macromolecules

- 3.3.1 Nucleic acid biosynthesis (DNA and RNA)
- 3.3.2 Protein biosynthesis and ribosomal function
- 3.3.3 Cell wall and membrane biosynthesis

3.4 Secondary Metabolites and Industrial Applications

- 3.4.1 Biosynthesis of antibiotics and other secondary metabolites
- 3.4.2 Microbial production of vitamins, pigments, and organic acids
- 3.4.3 Industrial applications of microbial biosynthetic processes

Introduction

In the last Series, we explored microbial nutrition, energy metabolism, and environmental factors, gaining insight into how micro-organisms obtain nutrients, generate energy, and adapt to their surroundings. Building on that foundation, this Series will take us deeper into the biochemical processes that drive microbial life, focusing on the molecular mechanisms of catalysis, metabolic pathways, and biosynthesis. These topics are central to understanding microbial physiology and their applications in biotechnology and industry.

The aim of this Series is to equip you with the ability to explain microbial enzymatic activity, analyse metabolic pathways, describe biosynthesis of macromolecules, and evaluate the production of secondary metabolites and their industrial significance.

We shall commence this Series by examining enzymes and catalysis in microbial systems, looking at their structure, function, and regulation. Next, we will explore metabolic pathways and biochemical cycles, including carbohydrate, lipid, and protein metabolism, and how these pathways integrate within microbial physiology. Following that, we will consider the biosynthesis of macromolecules such as nucleic acids, proteins, cell walls, and membranes.

Finally, we will wrap up by studying secondary metabolites and industrial applications, highlighting microbial production of antibiotics, vitamins, pigments, and organic acids, and their relevance to biotechnology. This progression will help you connect biochemical processes with practical applications in science and industry.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **3.1** define the structure and function of microbial enzymes,
- **3.2** explain the mechanisms of enzyme catalysis in microbial systems,
- **3.3** analyse how microbial enzyme activity is regulated,
- **3.4** describe carbohydrate metabolism, including glycolysis, the TCA cycle, and the pentose phosphate pathway,
- **3.5** explain lipid metabolism and outline the steps of fatty acid biosynthesis,
- **3.6** evaluate protein metabolism and amino acid biosynthesis in microbes,
- **3.7** assess how metabolic pathways integrate to support microbial physiology,
- **3.8** describe nucleic acid biosynthesis, including DNA and RNA synthesis,
- **3.9** explain protein biosynthesis and the role of ribosomes,
- **3.10** analyse cell wall and membrane biosynthesis in micro-organisms,
- **3.11** evaluate the biosynthesis of antibiotics and other secondary metabolites,
- **3.12** apply knowledge of microbial biosynthesis to discuss the production of vitamins, pigments, and organic acids, and
- **3.13** assess the industrial applications of microbial biosynthetic processes in biotechnology.

3.1 Enzymes And Catalysis In Microbial Systems

3.1.1 Structure and function of microbial enzymes

Micro-organisms rely on **enzymes**, defined as biological catalysts that speed up chemical reactions without being consumed in the process. Enzymes are typically proteins with specific three-dimensional structures that determine their activity. The region where the substrate binds is called the **active site**, and its shape complements the substrate, ensuring specificity.

Microbial enzymes are essential for processes such as nutrient breakdown, energy generation, and biosynthesis. For example, amylases degrade starch into sugars, while proteases break down proteins into amino acids.

Fill in the blank: The region of an enzyme where the substrate binds and the reaction occurs is called the _____.

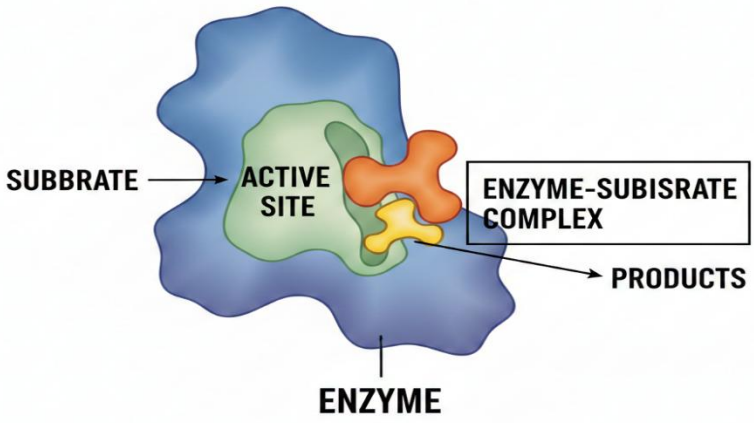


Figure 3.1 Structure and function of microbial enzymes

3.1.2 Mechanisms of enzyme catalysis

Enzymes catalyse reactions through several mechanisms. One is the **lock-and-key model**, where the substrate fits precisely into the active site. Another is the **induced fit model**, where the enzyme changes shape slightly to accommodate the substrate.

Catalysis often involves lowering the **activation energy**, defined as the minimum energy required for a reaction to occur. Enzymes achieve this by stabilising the transition state and bringing reactants into close proximity.

Fill in the blank: The minimum energy required for a chemical reaction to occur is called _____.

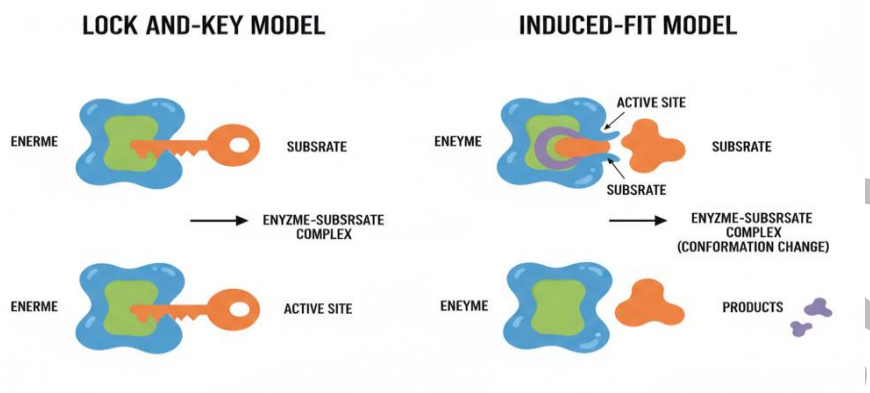


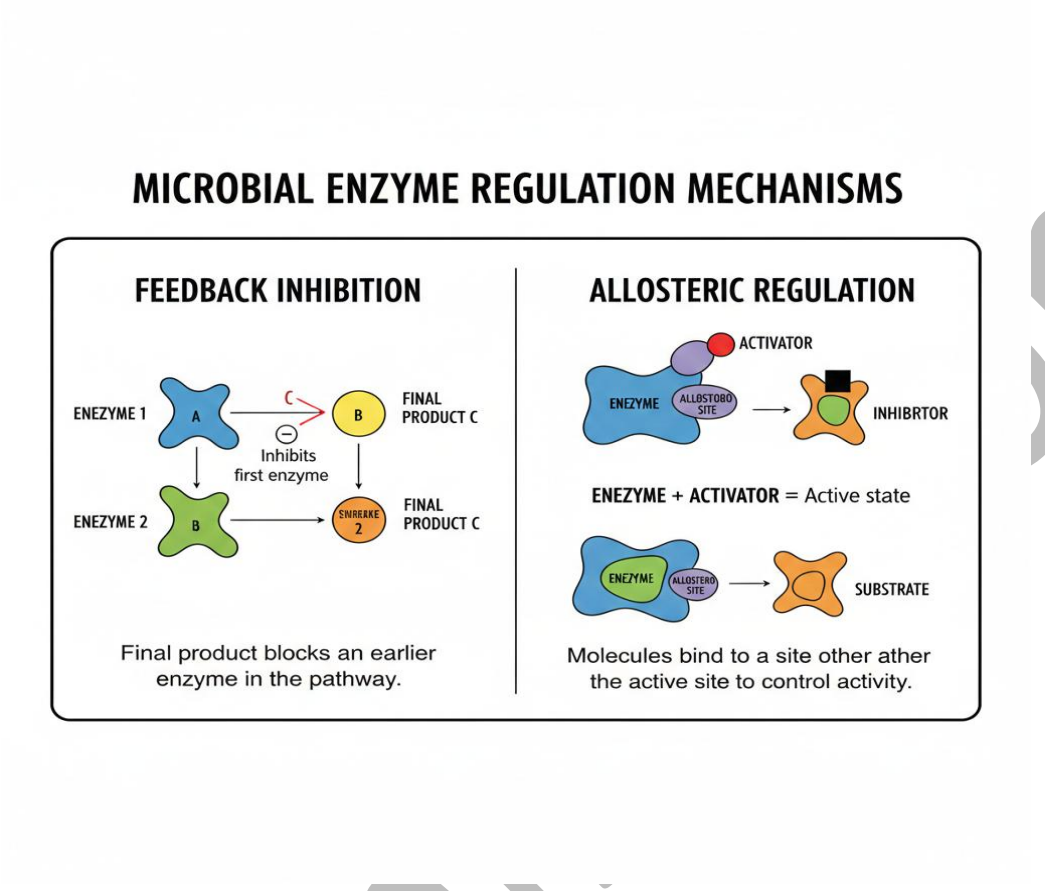
Figure 3.2 Mechanisms of enzyme catalysis

3.1.3 Regulation of enzyme activity in microbes

Microbes regulate enzyme activity to adapt to changing environments. One method is **feedback inhibition**, where the end product of a pathway inhibits an enzyme earlier in the pathway. Another is **allosteric regulation**, defined as the binding of molecules at sites other than the active site, which alters enzyme activity.

Enzyme activity can also be influenced by environmental factors such as temperature, pH, and substrate concentration. These regulatory mechanisms ensure that microbes conserve energy and resources while maintaining metabolic balance.

Fill in the blank: When the end product of a pathway inhibits an enzyme earlier in the pathway, the process is called _____.



Box 3.1 Regulation of enzyme activity in microbes

Pilot 3.1

1. The biological catalysts that speed up chemical reactions without being consumed are called _____.
2. The region of an enzyme where the substrate binds and the reaction occurs is called the _____.
3. The model of enzyme action where the enzyme changes shape slightly to fit the substrate is called the _____ model.
4. The minimum energy required for a chemical reaction to occur is called _____.
5. When the end product of a pathway inhibits an enzyme earlier in the pathway, the process is called _____.

3.2 Metabolic Pathways And Biochemical Cycles

3.2.1 Carbohydrate metabolism: glycolysis, TCA cycle, and pentose phosphate pathway

Micro-organisms rely heavily on **carbohydrate metabolism**, defined as the biochemical processes that break down or synthesise sugars to provide energy and precursors for biosynthesis. The most common pathway is **glycolysis**, where glucose is converted into pyruvate,

yielding ATP and NADH. Pyruvate then enters the **tricarboxylic acid (TCA) cycle**, also known as the Krebs cycle, which generates additional electron carriers (NADH and $FADH_2$) for energy production.

Another important pathway is the **pentose phosphate pathway**, which produces NADPH and ribose-5-phosphate. NADPH is essential for biosynthetic reactions, while ribose-5-phosphate is a precursor for nucleic acid synthesis.

Fill in the blank: The pathway that produces NADPH and ribose-5-phosphate for biosynthesis is called the _____.

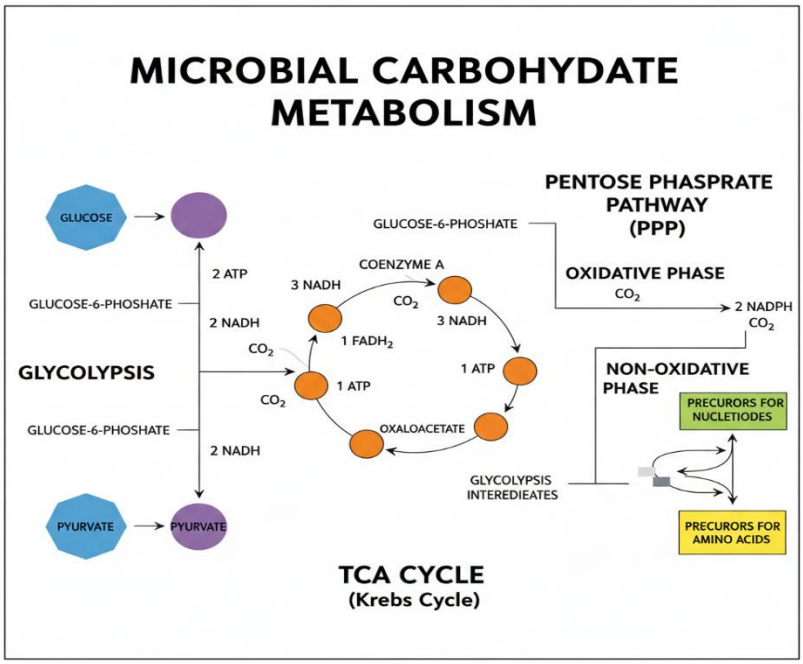


Figure 3.3 Carbohydrate metabolism in microbes

3.2.2 Lipid metabolism and fatty acid biosynthesis

Microbes also utilise **lipid metabolism**, defined as the breakdown and synthesis of fats for energy and structural purposes. Fatty acids can be degraded through **beta-oxidation**, producing acetyl-CoA that feeds into the TCA cycle.

In contrast, **fatty acid biosynthesis** involves the stepwise addition of two-carbon units to build long-chain fatty acids. These fatty acids are crucial for membrane structure and function, providing fluidity and integrity.

Fill in the blank: The process by which fatty acids are broken down into acetyl-CoA units is called _____.

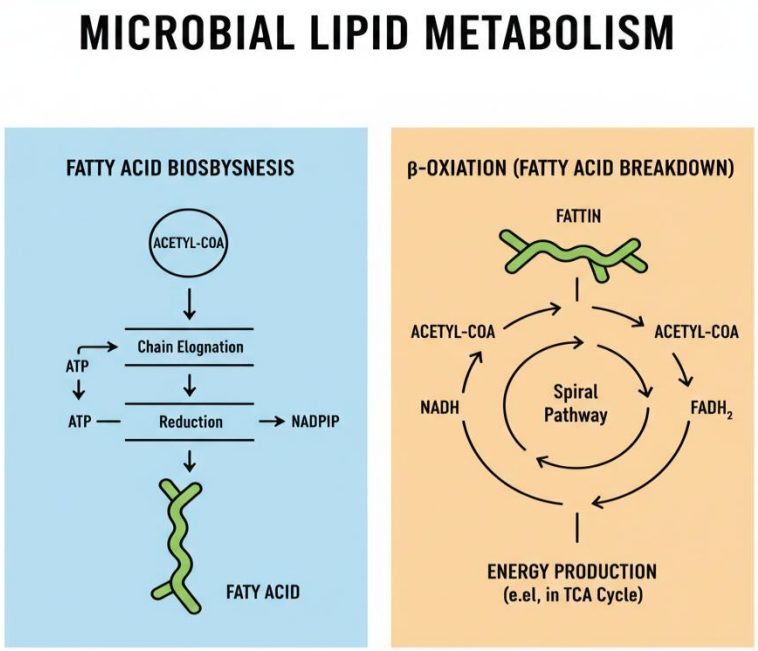


Figure 3.4 Lipid metabolism and fatty acid biosynthesis

3.2.3 Protein metabolism and amino acid biosynthesis

Microbes degrade proteins into amino acids through **proteolysis**, defined as the enzymatic breakdown of proteins. Amino acids can then be deaminated to produce intermediates that enter central metabolic pathways such as glycolysis or the TCA cycle.

Microbes also synthesise amino acids through **amino acid biosynthesis**, which involves pathways such as the synthesis of glutamate from alpha-ketoglutarate. These biosynthetic processes are essential for protein production and microbial growth.

Fill in the blank: The enzymatic breakdown of proteins into amino acids is called _____.

Amino Acid Family	Precursor(s)	Example Amino Acids	Metabolic Origin
Glutamate Family	α-ketoglutarate	Glutamate, Glutamine, Proline, Arginine	TCA Cycle
Aspartate Family	Oxaloacetate	Aspartate, Asparagine, Lysine, Threonine, Methionine	TCA Cycle
Serine Family	3-Phosphoglycerate	Serine, Glycine, Cysteine	Glycolysis

Amino Acid Family	Precursor(s)	Example Amino Acids	Metabolic Origin
Pyruvate Family	Pyruvate	Alanine, Valine, Leucine	Glycolysis
Aromatic Family	Phosphoenolpyruvate (PEP) & Erythrose-4-phosphate	Phenylalanine, Tyrosine, Tryptophan	Glycolysis / Pentose Phosphate Pathway
Histidine Family	Ribose-5-phosphate	Histidine	Pentose Phosphate Pathway

Table 3.1 Examples of amino acid biosynthesis pathways in microbes

3.2.4 Integration of metabolic pathways in microbial physiology

Microbial metabolism is highly interconnected. Carbohydrate, lipid, and protein pathways converge at central intermediates such as acetyl-CoA and pyruvate. This **integration of metabolic pathways**, defined as the coordination of different biochemical routes, ensures efficient energy use and balanced biosynthesis.

For example, acetyl-CoA derived from carbohydrates, lipids, or proteins can be channelled into the TCA cycle for energy or diverted into biosynthetic pathways for fatty acids and amino acids. This flexibility allows microbes to adapt to varying nutrient availability.

Fill in the blank: The central metabolic intermediate that links carbohydrate, lipid, and protein metabolism is _____.

Pathway Type	Connection to Acetyl-CoA	Key Processes
Carbohydrate Catabolism	**Input:** Pyruvate produced via Glycolysis is decarboxylated to form Acetyl-CoA.	Pyruvate dehydrogenase complex activity.
Lipid Metabolism	**Bidirectional:** Fatty acids are broken down into Acetyl-CoA; Acetyl-CoA is the building block for fatty acid synthesis.	β-oxidation and Fatty Acid Biosynthesis.
Energy Production	**Oxidation:** Acetyl-CoA enters the TCA cycle to generate reducing power and ATP.	Condensation with Oxaloacetate to form Citrate.
Protein Metabolism	**Input/Output:** Certain ketogenic amino acids are degraded into Acetyl-CoA.	Leucine, Lysine, and Tryptophan degradation.

Pathway Type	Connection to Acetyl-CoA	Key Processes
Secondary Metabolism	**Precursor:** Used to synthesize polyketides, pigments, and certain microbial toxins.	Specialized biosynthetic operons.

Box 3.2 Integration of metabolic pathways in microbial physiology

Pilot 3.2

1. The metabolic pathway that converts glucose into pyruvate while producing ATP is called _____.
2. The cycle that generates NADH and $FADH_2$ for energy production is the _____ cycle.
3. The pathway that produces NADPH and ribose-5-phosphate for biosynthesis is called the _____ pathway.
4. The process by which fatty acids are broken down into acetyl-CoA units is called _____.
5. The enzymatic breakdown of proteins into amino acids is called _____.
6. The central metabolic intermediate that links carbohydrate, lipid, and protein metabolism is _____.

3.3 Biosynthesis Of Macromolecules

3.3.1 Nucleic acid biosynthesis (DNA and RNA)

Micro-organisms synthesise **nucleic acids**, defined as macromolecules that store and transmit genetic information. DNA biosynthesis involves the replication of the double helix, where enzymes such as DNA polymerase add complementary nucleotides to form new strands. RNA biosynthesis, or transcription, is carried out by RNA polymerase, which produces messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

These processes are essential for cell division, protein synthesis, and the regulation of microbial activity. Without accurate nucleic acid biosynthesis, microbes cannot reproduce or maintain their genetic integrity.

Fill in the blank: The enzyme responsible for synthesising RNA from a DNA template is called _____.

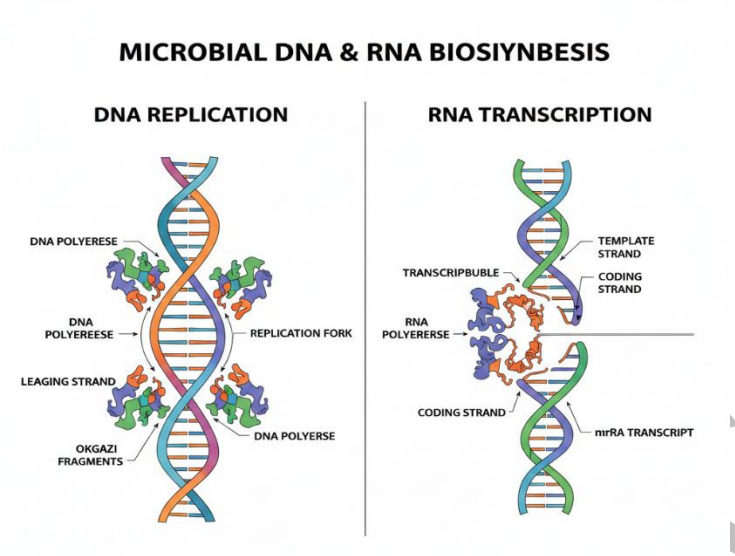


Figure 3.5 Nucleic acid biosynthesis in microbes

3.3.2 Protein biosynthesis and ribosomal function

Protein biosynthesis, defined as the process by which cells build proteins from amino acids, occurs in two stages: translation initiation and elongation. Ribosomes, composed of rRNA and proteins, serve as the sites of translation. Messenger RNA provides the template, while transfer RNA delivers amino acids to the ribosome.

The ribosome ensures that amino acids are joined in the correct sequence, producing functional proteins that carry out structural and enzymatic roles in the cell.

Fill in the blank: The cellular structures that serve as the sites of protein synthesis are called _____.

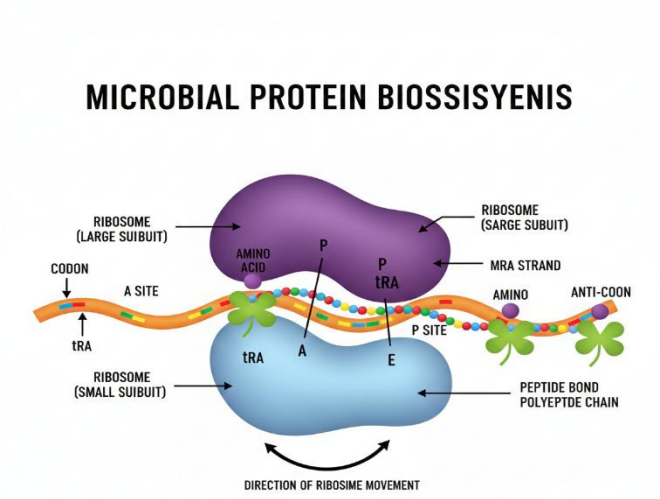


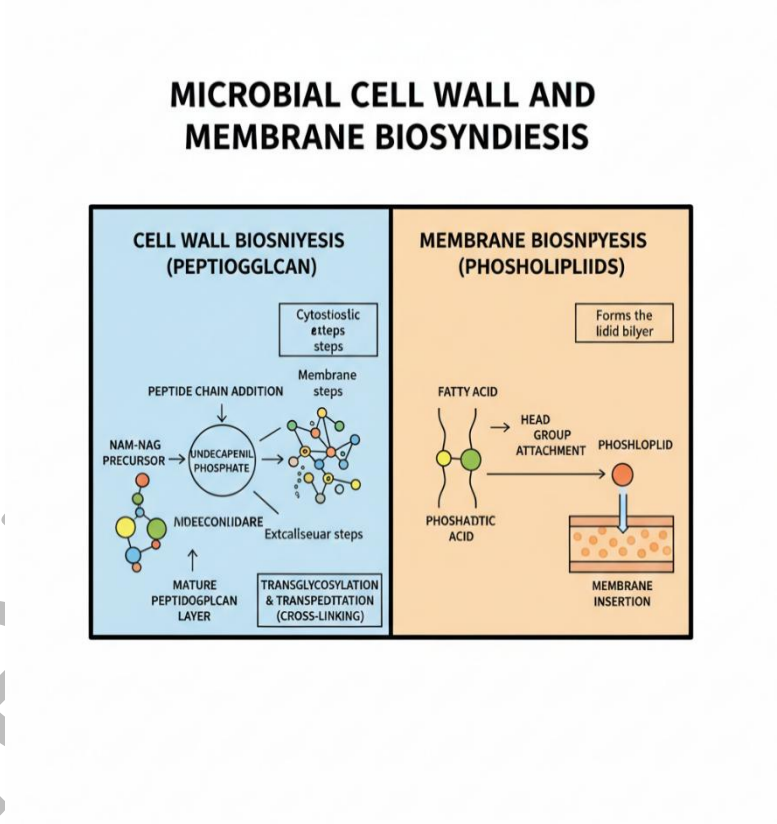
Figure 3.6 Protein biosynthesis and ribosomal function

3.3.3 Cell wall and membrane biosynthesis

Microbes synthesise **cell walls**, defined as rigid structures that provide shape and protection, and **membranes**, defined as flexible barriers that regulate transport and communication. In bacteria, cell wall biosynthesis involves the production of peptidoglycan, a polymer of sugars and amino acids. Enzymes such as transpeptidases link peptidoglycan strands, creating a strong mesh-like structure.

Membrane biosynthesis involves the assembly of phospholipids and proteins, which form bilayers that maintain fluidity and integrity. These structures are vital for microbial survival, as they protect against environmental stress and control nutrient exchange.

Fill in the blank: The polymer of sugars and amino acids that forms the bacterial cell wall is called _____.



Box 3.3 Biosynthesis of microbial cell walls and membranes

Pilot 3.3

1. The enzyme responsible for synthesising RNA from a DNA template is called _____.
2. The cellular structures that serve as the sites of protein synthesis are called _____.
3. The polymer of sugars and amino acids that forms the bacterial cell wall is called _____.

3.4 Secondary Metabolites And Industrial Applications

3.4.1 Biosynthesis of antibiotics and other secondary metabolites

Micro-organisms produce **secondary metabolites**, defined as compounds not directly involved in growth or reproduction but often providing ecological advantages. A key example is **antibiotics**, chemical substances that inhibit or kill other microbes. These are typically synthesised during the stationary phase of growth when nutrients become limited.

Secondary metabolites also include toxins, signalling molecules, and pigments that help microbes compete or adapt to their environment. Their biosynthesis often involves specialised pathways such as polyketide or non-ribosomal peptide synthesis.

Fill in the blank: Chemical substances produced by microbes that inhibit or kill other organisms are called _____.

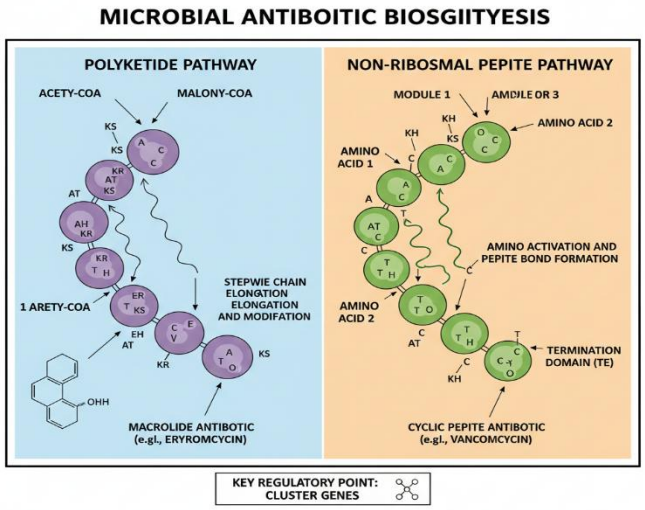


Figure 3.7 Biosynthesis of antibiotics and secondary metabolites

3.4.2 Microbial production of vitamins, pigments, and organic acids

Microbes are widely used in the production of **vitamins**, defined as organic compounds required in small amounts for normal metabolism. For example, bacteria such as Propionibacterium produce vitamin B_{12}.

They also synthesise **pigments**, which are coloured compounds that can serve protective or commercial roles. Carotenoids and melanin are examples of microbial pigments with industrial applications.

Additionally, microbes produce **organic acids** such as citric acid, lactic acid, and acetic acid. These acids are used in food preservation, pharmaceuticals, and chemical industries.

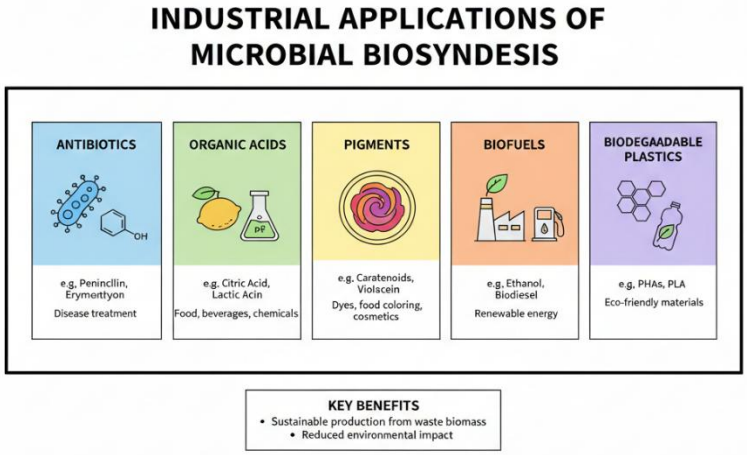
Fill in the blank: The bacterium Propionibacterium is commonly used in the microbial production of vitamin _____.

Category	Product	Example Organism(s)	Primary Industrial Use
Vitamins	**Vitamin B_{12}** (Cobalamin)	*Pseudomonas denitrificans*, *Propionibacterium freudenreichii*	Treatment of anemia, food fortification.
	Vitamin B_{2} (Riboflavin)	*Ashbya gossypii*, *Bacillus subtilis*	Animal feed additive, food coloring.
	Vitamin C (Ascorbic acid)	*Gluconobacter oxydans*, *Ketogulonicigenium vulgare*	Antioxidant, dietary supplement.
	β-carotene (Pro-vitamin A)	*Blakeslea trispora*, *Dunaliella salina* (alga)	Food coloring, antioxidant.
Pigments	**Prodigiosin** (Red)	*Serratia marcescens*	Natural dye, antimicrobial agent.
	Violacein (Purple)	*Chromobacterium violaceum*	Textile dyeing, anti-tumor research.
	Astaxanthin (Pink-Red)	*Haematococcus pluvialis* (alga), *Paracoccus* spp.	Aquaculture (salmon color), nutraceuticals.
	Pyocyanin (Blue-Green)	*Pseudomonas aeruginosa*	Redox-active research, biosensors.
Organic Acids	**Citric Acid**	*Aspergillus niger* (fungus)	Acidulant in beverages, preservative.
	Lactic Acid	*Lactobacillus* spp., *Rhizopus oryzae*	Food preservation, bioplastics (PLA).
	Acetic Acid	*Acetobacter aceti*	Vinegar production, chemical precursor.
	Itaconic Acid	*Aspergillus terreus*	Manufacture of resins and synthetic fibers.

Table 3.2 Examples of microbial production of vitamins, pigments, and organic acids

3.4.3 Industrial applications of microbial biosynthetic processes

The biosynthesis of secondary metabolites has extensive **industrial applications**, defined as practical uses of microbial processes in manufacturing and biotechnology. Antibiotics are used in medicine, organic acids in food and chemical industries, and pigments in cosmetics and textiles. Microbial biosynthetic processes also contribute to sustainable practices, such as producing biodegradable plastics and biofuels. These applications highlight the economic and environmental importance of microbial biochemistry.
Fill in the blank: The microbial production of biodegradable plastics and biofuels represents an example of _____ applications.



Box 3.4 Industrial applications of microbial biosynthetic processes

Pilot Questions 3.4

1. Compounds produced by microbes that are not essential for growth but provide ecological advantages are called _____.
2. Chemical substances produced by microbes that inhibit or kill other organisms are called _____.
3. The bacterium Propionibacterium is commonly used in the microbial production of vitamin _____.
4. Carotenoids and melanin are examples of microbial _____.
5. Citric acid, lactic acid, and acetic acid are examples of microbial _____.
6. The microbial production of biodegradable plastics and biofuels represents an example of _____ applications.

Series Summary

This Series has guided you through the **biochemistry of microbial processes and biosynthesis**, showing how micro-organisms carry out complex biochemical reactions that sustain life and drive industrial applications. We began with enzymes and catalysis, examining their structure, function, and regulation. Then we explored metabolic pathways and biochemical cycles, including carbohydrate, lipid, and protein metabolism, and how these pathways integrate to support microbial physiology. Following that, we studied the biosynthesis of macromolecules such as nucleic acids, proteins, cell walls, and membranes. Finally, we considered secondary metabolites and their industrial applications, highlighting microbial production of antibiotics, vitamins, pigments, organic acids, and sustainable products like biofuels and biodegradable plastics.

By completing this Series, you should now be able to define microbial enzymes and explain their catalytic mechanisms, analyse metabolic pathways and evaluate their integration, describe the biosynthesis of macromolecules, and assess the production and applications of secondary metabolites. In line with the Series Learning Outcomes, you are equipped to connect biochemical processes with practical applications in biotechnology and industry, reinforcing the relevance of microbial biochemistry to both science and society.

Glossary of Terms

- **Activation energy**: The minimum amount of energy required for a chemical reaction to occur.
- **Active site**: The region of an enzyme where the substrate binds and the reaction takes place.
- **Allosteric regulation**: Control of enzyme activity by molecules binding at sites other than the active site, altering the enzyme's function.
- **Amino acid biosynthesis**: The process by which micro-organisms produce amino acids from metabolic precursors.
- **Antibiotics**: Chemical substances produced by microbes that inhibit or kill other organisms.
- **Beta-oxidation**: The metabolic process that breaks down fatty acids into acetyl-CoA units.
- **Carbohydrate metabolism**: Biochemical processes that break down or synthesise sugars to provide energy and precursors for biosynthesis.
- **Cell wall**: A rigid structure composed mainly of peptidoglycan in bacteria, providing shape and protection.
- **Enzymes**: Biological catalysts that speed up chemical reactions without being consumed.
- **Feedback inhibition**: Regulation where the end product of a pathway inhibits an enzyme earlier in the pathway.
- **Glycolysis**: A metabolic pathway that converts glucose into pyruvate, producing ATP and NADH.
- **Induced fit model**: A model of enzyme action where the enzyme changes shape slightly to fit the substrate.
- **Nucleic acids**: Macromolecules (DNA and RNA) that store and transmit genetic information.

- **Organic acids**: Compounds such as citric acid, lactic acid, and acetic acid produced by microbes for industrial use.
- **Pentose phosphate pathway**: A metabolic pathway that produces NADPH and ribose-5-phosphate for biosynthesis.
- **Pigments**: Coloured compounds produced by microbes, often serving protective or commercial roles.
- **Proteolysis**: The enzymatic breakdown of proteins into amino acids.
- **Protein biosynthesis**: The process by which cells build proteins from amino acids, occurring at ribosomes.
- **Ribosome**: A cellular structure composed of rRNA and proteins, serving as the site of protein synthesis.
- **Secondary metabolites**: Compounds not essential for growth but providing ecological or competitive advantages.
- **TCA cycle (Krebs cycle)**: A central metabolic cycle that generates NADH and $FADH_2$ for energy production.

Concept Check Questions [Series 3: Biochemistry of Microbial Processes and Biosynthesis]

Objective questions

1. Which enzyme synthesises RNA from a DNA template? (Linked to SLO 3.8)
   a) DNA polymerase
   b) RNA polymerase
   c) ATP synthase
   d) Ligase
2. The metabolic pathway that produces NADPH and ribose-5-phosphate is: (Linked to SLO 3.4)
   a) Glycolysis
   b) TCA cycle
   c) Pentose phosphate pathway
   d) Beta-oxidation
3. The bacterial cell wall is primarily composed of: (Linked to SLO 3.10)
   a) Cellulose
   b) Peptidoglycan
   c) Chitin
   d) Lipopolysaccharides
4. Which microbial product is widely used in food preservation and chemical industries? (Linked to SLO 3.12)
   a) Antibiotics
   b) Organic acids
   c) Pigments
   d) Vitamins

Short-answer questions

1. Define enzymes and explain their role in microbial metabolism. (Linked to SLO 3.1)
2. Describe the difference between the lock-and-key model and the induced fit model of enzyme catalysis. (Linked to SLO 3.2)
3. State one example of microbial pigment and its industrial application. (Linked to SLO 3.12)
4. Explain how feedback inhibition regulates enzyme activity. (Linked to SLO 3.3)
5. What is the central metabolic intermediate that links carbohydrate, lipid, and protein metabolism? (Linked to SLO 3.7)

Long-answer questions

1. Analyse carbohydrate metabolism in microbes, focusing on glycolysis, the TCA cycle, and the pentose phosphate pathway. (Linked to SLO 3.4)
2. Evaluate the biosynthesis of macromolecules in microbes, including nucleic acids, proteins, and cell walls. (Linked to SLO 3.8, SLO 3.9, SLO 3.10)
3. Assess the industrial applications of microbial biosynthetic processes, with examples of antibiotics, organic acids, pigments, and biofuels. (Linked to SLO 3.11, SLO 3.12, SLO 3.13)

Answers to Concept Check Questions [Series 3: Biochemistry of Microbial Processes and Biosynthesis]

Objective questions

1. b) RNA polymerase
2. c) Pentose phosphate pathway
3. b) Peptidoglycan
4. b) Organic acids

Short-answer questions

1. Enzymes are biological catalysts that speed up chemical reactions without being consumed. They are essential for microbial metabolism, enabling nutrient breakdown and biosynthesis.
2. The lock-and-key model suggests substrates fit precisely into the enzyme's active site, while the induced fit model proposes that the enzyme changes shape slightly to accommodate the substrate.
3. Carotenoids are microbial pigments used in food colouring and cosmetics.
4. Feedback inhibition occurs when the end product of a pathway inhibits an enzyme earlier in the pathway, preventing overproduction.
5. Acetyl-CoA is the central metabolic intermediate linking carbohydrate, lipid, and protein metabolism.

Long-answer questions

1. ***Basic response*:** Glycolysis converts glucose into pyruvate, producing ATP and NADH. The TCA cycle generates NADH and $FADH_2$ for energy, while the pentose phosphate pathway produces NADPH and ribose-5-phosphate for biosynthesis.
   ***Value-added response*:** These pathways are interconnected, allowing microbes to balance energy production with biosynthetic needs. The pentose phosphate pathway also supports antioxidant defence through NADPH.
2. ***Basic response*:** Nucleic acid biosynthesis involves DNA replication and RNA transcription. Protein biosynthesis occurs at ribosomes using mRNA templates. Cell wall biosynthesis involves peptidoglycan assembly for structural integrity.
   ***Value-added response*:** These processes are tightly regulated, ensuring accurate genetic transmission, efficient protein production, and robust cell wall formation. They are also targets for antibiotics, highlighting their medical relevance.
3. ***Basic response*:** Microbial biosynthesis produces antibiotics for medicine, organic acids for food and chemical industries, pigments for cosmetics, and biofuels for energy.
   ***Value-added response*:** Beyond these uses, microbial biosynthesis supports sustainable practices such as biodegradable plastics, reducing reliance on fossil fuels and contributing to environmental conservation.

Answers to Pilot Questions [Series 3: Biochemistry of Microbial Processes and Biosynthesis]

Part 3.1 Enzymes and Catalysis in Microbial Systems

1. Enzymes
2. Active site
3. Induced fit model
4. Activation energy
5. Feedback inhibition

Part 3.2 Metabolic Pathways and Biochemical Cycles

1. Glycolysis
2. TCA cycle (Krebs cycle)
3. Pentose phosphate pathway
4. Beta-oxidation
5. Proteolysis
6. Acetyl-CoA

Part 3.3 Biosynthesis of Macromolecules

1. RNA polymerase
2. Ribosomes
3. Peptidoglycan

Part 3.4 Secondary Metabolites and Industrial Applications

1. Secondary metabolites
2. Antibiotics
3. Vitamin B_{12}
4. Pigments
5. Organic acids
6. Industrial applications

References and Attributions [Series 3: Biochemistry of Microbial Processes and Biosynthesis]

Figures

- Figure 3.1 Structure and function of microbial enzymes. AI-generated using the prompt: "Generate a labelled diagram showing enzyme structure with active site and substrate binding." Suggested OER source: "OER enzyme structure active site diagram."
- Figure 3.2 Mechanisms of enzyme catalysis. AI-generated using the prompt: "Generate a labelled diagram comparing lock-and-key and induced fit models of enzyme catalysis." Suggested OER source: "OER enzyme catalysis lock-and-key induced fit diagram."
- Figure 3.3 Carbohydrate metabolism in microbes. AI-generated using the prompt: "Generate a labelled diagram showing glycolysis, TCA cycle, and pentose phosphate pathway in microbes with their products." Suggested OER source: "OER carbohydrate metabolism glycolysis TCA pentose phosphate diagram."
- Figure 3.4 Lipid metabolism and fatty acid biosynthesis. AI-generated using the prompt: "Generate a labelled diagram showing fatty acid biosynthesis and beta-oxidation in microbes." Suggested OER source: "OER lipid metabolism fatty acid biosynthesis diagram."
- Figure 3.5 Nucleic acid biosynthesis in microbes. AI-generated using the prompt: "Generate a labelled diagram showing DNA replication and RNA transcription in microbes with DNA polymerase and RNA polymerase." Suggested OER source: "OER DNA RNA biosynthesis diagram microbiology."
- Figure 3.6 Protein biosynthesis and ribosomal function. AI-generated using the prompt: "Generate a labelled diagram showing protein biosynthesis at the ribosome with mRNA, tRNA, and amino acids." Suggested OER source: "OER protein biosynthesis ribosome diagram."
- Figure 3.7 Biosynthesis of antibiotics and secondary metabolites. AI-generated using the prompt: "Generate a labelled diagram showing microbial biosynthesis of antibiotics via polyketide and non-ribosomal peptide pathways." Suggested OER source: "OER microbial biosynthesis antibiotics secondary metabolites diagram."

Tables

- Table 3.1 Examples of amino acid biosynthesis pathways in microbes. AI-generated using the prompt: "Generate a table showing examples of amino acid biosynthesis pathways in microbes with precursors." Suggested OER source: "OER amino acid biosynthesis pathways table."

- Table 3.2 Examples of microbial production of vitamins, pigments, and organic acids. AI-generated using the prompt: “Generate a table showing microbial production of vitamins, pigments, and organic acids with examples of organisms.” Suggested OER source: “OER microbial production vitamins pigments organic acids table.”

Boxes

- Box 3.1 Regulation of enzyme activity in microbes. AI-generated using the prompt: “Generate a box summarising microbial enzyme regulation mechanisms including feedback inhibition and allosteric regulation.” Suggested OER source: “OER enzyme regulation feedback inhibition allosteric control.”
- Box 3.2 Integration of metabolic pathways in microbial physiology. AI-generated using the prompt: “Generate a box summarising integration of metabolic pathways in microbes with acetyl-CoA as a central intermediate.” Suggested OER source: “OER integration of metabolic pathways microbes.”
- Box 3.3 Biosynthesis of microbial cell walls and membranes. AI-generated using the prompt: “Generate a box summarising microbial cell wall and membrane biosynthesis including peptidoglycan and phospholipids.” Suggested OER source: “OER microbial cell wall membrane biosynthesis.”
- Box 3.4 Industrial applications of microbial biosynthetic processes. AI-generated using the prompt: “Generate a box summarising industrial applications of microbial biosynthetic processes including antibiotics, organic acids, pigments, biofuels, and biodegradable plastics.” Suggested OER source: “OER industrial applications microbial biosynthesis.”

Textual references

- Explanations of microbial enzymes, metabolic pathways, biosynthesis of macromolecules, and secondary metabolites were derived from the instructional content developed for Series 3.
- Standard microbiology references on enzyme catalysis, glycolysis, TCA cycle, pentose phosphate pathway, nucleic acid and protein biosynthesis, and microbial ecology informed the descriptions of biochemical processes.
- Definitions of key terms such as enzymes, activation energy, peptidoglycan, antibiotics, pigments, and secondary metabolites were aligned with widely accepted microbiology textbooks and open educational resources.

Course Code: MCB 424
Course Title: Microbial Physiology & Metabolism
Course Unit: 3 Units

Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems
Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors
Series 3: Biochemistry of Microbial Processes and Biosynthesis
Series 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

Series 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Series 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

Series Outline

Part 4.1 Fundamentals of Bioenergetics

- **4.1.1 Energy in Biological Systems**: Definition of energy, forms of energy relevant to microbial physiology.
- **4.1.2 Thermodynamics in Microbial Metabolism**: Laws of thermodynamics, free energy, and entropy.
- **4.1.3 ATP and Energy Coupling**: Role of adenosine triphosphate (ATP), phosphorylation, and energy transfer.

Part 4.2 Enzyme Structure and Function

- **4.2.1 Properties of Enzymes**: Definition of enzymes, catalytic power, specificity.
- **4.2.2 Enzyme Kinetics**: Michaelis-Menten model, Km and Vmax, factors affecting enzyme activity.
- **4.2.3 Enzyme Regulation**: Allosteric control, feedback inhibition, covalent modification.

Part 4.3 Metabolic Pathways and Integration

- **4.3.1 Catabolic Pathways**: Glycolysis, tricarboxylic acid (TCA) cycle, fermentation.
- **4.3.2 Anabolic Pathways**: Biosynthesis of amino acids, nucleotides, and lipids.
- **4.3.3 Amphibolic Pathways**: Pathways serving both catabolic and anabolic roles.
- **4.3.4 Metabolic Flux and Control**: Regulation of pathway direction and rate.

Part 4.4 Specialised Bioenergetic Processes in Microbes

- **4.4.1 Electron Transport Chains**: Components, organisation, and microbial diversity.
- **4.4.2 Oxidative Phosphorylation**: Proton motive force, chemiosmosis, ATP synthase.
- **4.4.3 Alternative Energy Sources**: Phototrophy, lithotrophy, and anaerobic respiration.

Introduction

Microbial life depends on the ability to capture, transform, and utilise energy efficiently. Without energy, cells cannot grow, reproduce, or maintain their internal organisation. In microbes, energy flow is closely linked to enzyme action and the organisation of metabolic pathways. This Series will help you appreciate how microorganisms harness energy from diverse sources, how enzymes act as biological catalysts, and how metabolic pathways are integrated to sustain life. The aim of this Series is to equip you with the knowledge and skills to explain the principles of bioenergetics, analyse enzyme function and regulation, and evaluate the organisation of microbial metabolic pathways.

We shall commence this Series by examining the fundamentals of bioenergetics, including the role of energy in biological systems, thermodynamic principles, and ATP as the universal energy currency. Next, we will explore enzyme structure and function, focusing on their properties, kinetics, and regulation. Following that, we will move into metabolic pathways, considering catabolic, anabolic, and amphibolic processes, as well as how metabolic flux is controlled. Finally, we will conclude with specialised bioenergetic processes in microbes, such as electron transport chains, oxidative phosphorylation, and alternative energy sources like phototrophy and lithotrophy. This sequential approach will provide you with a comprehensive understanding of how microbes sustain their physiology through energy and metabolism.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **4.1** define the concept of energy in biological systems and explain its relevance to microbial physiology,
- **4.2** analyse the principles of thermodynamics as they apply to microbial metabolism,
- **4.3** explain the role of ATP in energy coupling and evaluate its function as the universal energy currency,
- **4.4** describe the structural and functional properties of enzymes,
- **4.5** demonstrate how enzyme kinetics are represented using the Michaelis-Menten model,
- **4.6** evaluate mechanisms of enzyme regulation including allosteric control and feedback inhibition,
- **4.7** explain the organisation of catabolic pathways such as glycolysis, the TCA cycle, and fermentation,
- **4.8** describe anabolic pathways involved in the biosynthesis of amino acids, nucleotides, and lipids,
- **4.9** analyse amphibolic pathways and assess their dual roles in metabolism,
- **4.10** evaluate metabolic flux and control in microbial systems,
- **4.11** describe the components and organisation of microbial electron transport chains,
- **4.12** explain the process of oxidative phosphorylation and the role of the proton motive force, and
- **4.13** compare alternative microbial energy sources such as phototrophy, lithotrophy, and anaerobic respiration.

4.1 Fundamentals of Bioenergetics

4.1.1 Energy in biological systems

All living organisms require **energy**, which is defined as the capacity to do work. In microbial physiology, energy is essential for processes such as growth, reproduction, motility, and maintenance of cellular structures. Microbes obtain energy from their environment and convert it into usable forms to sustain life. The sources of energy may vary widely, ranging from light in photosynthetic organisms to chemical compounds in heterotrophs.

Energy in biological systems exists in different forms, including chemical energy stored in bonds, kinetic energy associated with movement, and potential energy stored in gradients across membranes. Microbes are particularly adept at exploiting diverse energy sources, which explains their ability to thrive in varied environments.

Fill in the blank: Energy is defined as the capacity to _____.

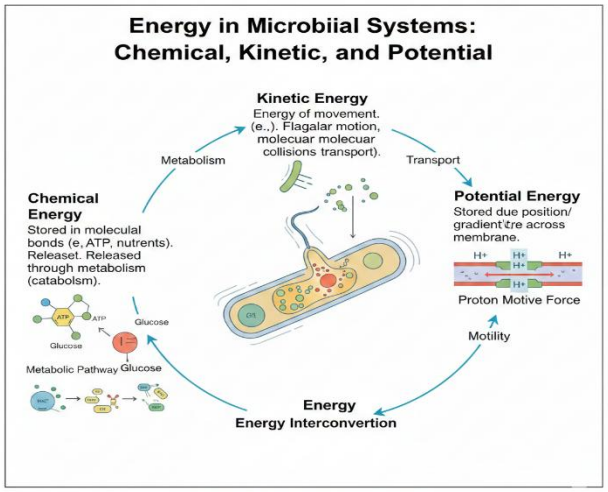


Figure 4.1 Forms of energy relevant to microbial physiology.

4.1.2 Thermodynamics in microbial metabolism

The study of **thermodynamics** involves the principles governing energy transformations. The **first law of thermodynamics** states that energy cannot be created or destroyed, only transformed from one form to another. The **second law of thermodynamics** states that every energy transfer increases the entropy, or disorder, of the universe.

In microbial metabolism, these laws explain how energy is conserved and why cells must continually acquire energy to maintain order. The concept of **free energy (ΔG)** is central here. Free energy refers to the portion of energy available to do work. A negative ΔG indicates a spontaneous reaction, while a positive ΔG requires energy input.

Fill in the blank: The second law of thermodynamics states that every energy transfer increases _____.

Law	Core Concept	Microbial Application & Example
First Law (Energy Conservation)	Energy cannot be created or destroyed, only transformed from one form to another.	**Application:** Microbes act as energy transducers, converting chemical or light energy into cellular work. **Example:** A chemolithotroph oxidizes ammonia (NH_3) to nitrite (NO_2^-), capturing the released chemical energy to build ATP.
Second Law (Entropy)	In every energy transfer, some energy is lost as heat, increasing the total disorder (entropy) of the universe.	**Application:** No metabolic process is 100% efficient; growth always generates heat and metabolic byproducts.

Law	Core Concept	Microbial Application & Example
		Example: During fermentation, only a fraction of the glucose energy ends up in ATP; the rest is lost as heat, warming the surrounding culture media.

Box 4.1 Key thermodynamic principles in microbial metabolism

4.1.3 ATP and energy coupling

Microbial cells rely on **adenosine triphosphate (ATP)**, which is defined as the universal energy currency of the cell. ATP stores energy in its high-energy phosphate bonds. When these bonds are broken, energy is released and used to drive cellular processes.

The process of **energy coupling** refers to the use of energy released from exergonic reactions (those that release energy) to power endergonic reactions (those that require energy). For example, the breakdown of glucose in glycolysis releases energy, which is then used to synthesise ATP. ATP in turn fuels biosynthetic reactions, motility, and active transport.

Fill in the blank: ATP is often referred to as the universal _____ currency of the cell.

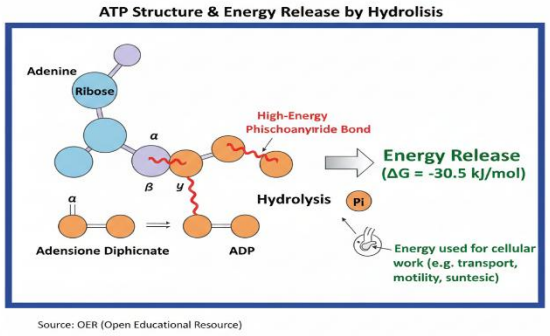


Figure 4.2 Structure of ATP and its role in energy transfer.

Pilot questions 4.1

1. Which form of energy is stored in chemical bonds?
2. State the first law of thermodynamics.
3. What does a negative ΔG indicate about a reaction?
4. What molecule is considered the universal energy currency of the cell?
5. Define energy coupling in microbial metabolism.

4.2 Enzyme Structure and Function

4.2.1 Properties of enzymes

Enzymes are biological catalysts, defined as proteins that speed up chemical reactions without being consumed in the process. They lower the activation energy required for reactions, making metabolic processes proceed at rates compatible with life. Enzymes are highly specific, meaning each enzyme acts on a particular substrate or group of substrates. This specificity is often described using the lock-and-key model, where the enzyme's active site fits the substrate precisely.

Enzymes also exhibit remarkable catalytic power, enabling reactions that would otherwise occur very slowly. Their activity can be influenced by environmental conditions such as temperature and pH.

Fill in the blank: Enzymes are biological _____ that speed up chemical reactions.

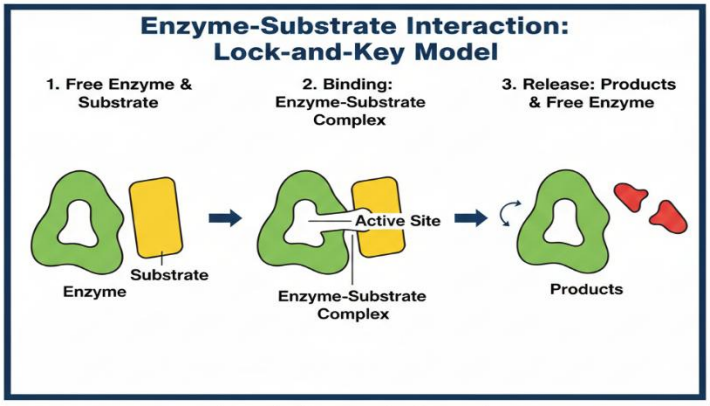


Figure 4.3 Enzyme-substrate interaction in the lock-and-key model.

4.2.2 Enzyme kinetics

Enzyme kinetics refers to the study of the rates of enzyme-catalysed reactions. The most widely used model is the **Michaelis-Menten equation**, which describes how reaction velocity depends on substrate concentration. Two key parameters are important: **Km**, the substrate concentration at which the reaction rate is half of its maximum, and **Vmax**, the maximum rate achieved when the enzyme is saturated with substrate.

Factors such as enzyme concentration, substrate concentration, temperature, and pH can influence reaction rates. Enzyme kinetics provides insights into how enzymes function under different conditions and how inhibitors affect their activity.

Fill in the blank: The substrate concentration at which the reaction rate is half of its maximum is called _____.

```
Pythonimport numpy as npimport matplotlib.pyplot as plt
# Define the Michaelis-Menten equationdef michaelis_menten(S, Vmax, Km):
    return (Vmax * S) / (Km + S)
# Parameters
Vmax_val = 10
Km_val = 2
S = np.linspace(0, 20, 500)
v = michaelis_menten(S, Vmax_val, Km_val)
# Plotting
plt.figure(figsize=(10, 7))
plt.plot(S, v, 'b-', linewidth=3, label='Reaction Velocity ($v$)')
# Add Vmax line and label
plt.axhline(y=Vmax_val, color='r', linestyle='--', linewidth=1.5)
plt.text(20.5, Vmax_val, '$V_{max}$', color='red', fontsize=14, va='center')
# Add Km and Vmax/2 annotations
half_vmax = Vmax_val / 2
plt.axhline(y=half_vmax, xmin=0, xmax=Km_val/20, color='gray', linestyle=':', linewidth=1.5)
plt.axvline(x=Km_val, ymin=0, ymax=half_vmax/Vmax_val, color='gray', linestyle=':', linewidth=1.5)
# Highlight Km point
plt.scatter([Km_val], [half_vmax], color='black', zorder=5)
# Labels for Km and Vmax/2
plt.text(Km_val, -0.6, '$K_m$', color='black', fontsize=14, ha='center')
plt.text(-1.5, half_vmax, '$\\frac{1}{2}V_{max}$', color='black', fontsize=14, va='center')
# Axis labels and title
plt.xlabel('Substrate Concentration $[S]$', fontsize=14)
plt.ylabel('Reaction Velocity $v$', fontsize=14)
plt.title('Michaelis-Menten Kinetics', fontsize=16)
# Formatting
plt.xlim(0, 20)
plt.ylim(0, Vmax_val * 1.1)
plt.grid(True, linestyle='--', alpha=0.5)
plt.tight_layout()
# Save image
plt.savefig('michaelis_menten_final.png')
plt.close()
```

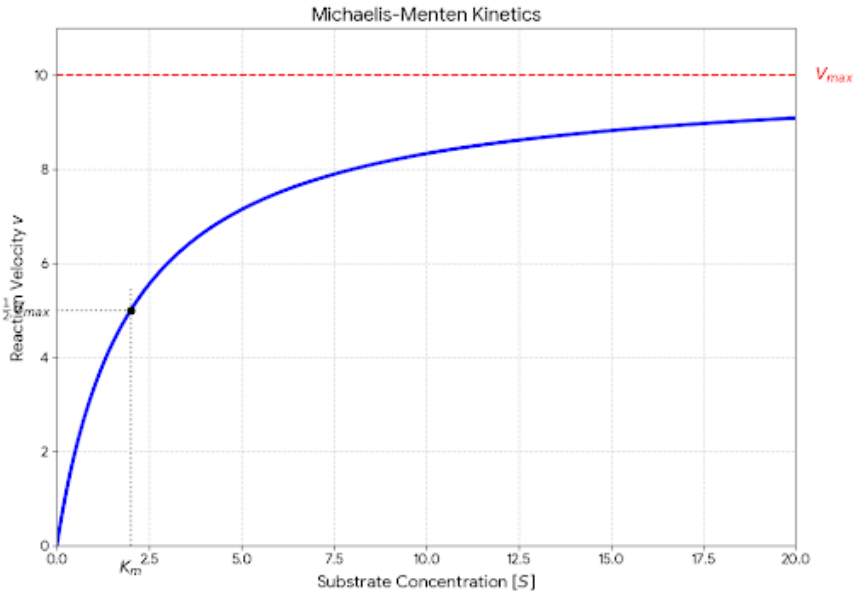


Figure 4.4 Michaelis-Menten curve showing Km and Vmax.

4.2.3 Enzyme regulation

Enzyme regulation refers to the mechanisms by which cells control enzyme activity to maintain metabolic balance. One common mechanism is **allosteric regulation**, where molecules bind to sites other than the active site, changing enzyme activity. Another is **feedback inhibition**, where the end product of a pathway inhibits an enzyme earlier in the pathway, preventing overproduction. Enzymes can also be regulated through **covalent modification**, such as phosphorylation, which alters their activity.

These regulatory mechanisms ensure that metabolic pathways operate efficiently and respond to changes in cellular needs.

Fill in the blank: When the end product of a pathway inhibits an enzyme earlier in the pathway, this is called _____ inhibition.

Mechanism	Description	Example
Allosteric Regulation	Molecules bind to a site other than the active site (allosteric site), causing a conformational change that either activates or inhibits the enzyme.	**ATP** inhibiting **Phosphofructokinase** in glycolysis (feedback inhibition).
Reversible Covalent Modification	The addition or removal of a chemical group (most commonly a phosphate) alters the enzyme's activity.	**Glycogen phosphorylase** is activated by the addition of a phosphate group (phosphorylation).

Mechanism	Description	Example
Competitive Inhibition	An inhibitor molecule resembles the substrate and competes for binding at the active site.	**Statins** competing with HMG-CoA for the active site of HMG-CoA reductase to lower cholesterol.
Non-competitive Inhibition	An inhibitor binds to a different site, changing the enzyme's shape so the substrate can no longer bind or be processed effectively.	**Cyanide** binding to mitochondrial cytochrome oxidase, halting the electron transport chain.
Zymogen Activation	Enzymes are synthesized in an inactive form (proenzymes) and activated by selective cleavage of peptide bonds.	**Pepsinogen** being converted into the active protease **Pepsin** by stomach acid (HCl).
Feedback Inhibition	The final product of a metabolic pathway acts as an inhibitor for an enzyme earlier in the same pathway.	**Isoleucine** inhibiting threonine deaminase to prevent its own overproduction.

Box 4.2 Mechanisms of enzyme regulation

Pilot questions 4.2

1. Define enzymes and state their role in metabolism.
2. What does the lock-and-key model illustrate about enzyme specificity?
3. In enzyme kinetics, what does Km represent?
4. What is the maximum rate of an enzyme-catalysed reaction called?
5. Name two mechanisms of enzyme regulation.

4.3 Metabolic Pathways and Integration

4.3.1 Catabolic pathways

Catabolism is defined as the set of metabolic processes that break down complex molecules into simpler ones, releasing energy in the process. In microbes, catabolic pathways provide both energy and precursor molecules for biosynthesis. The most important catabolic pathway is **glycolysis**, which breaks down glucose into pyruvate while generating ATP and NADH. The **tricarboxylic acid (TCA) cycle** further oxidises pyruvate to carbon dioxide, producing additional energy carriers. In the absence of oxygen, microbes may rely on **fermentation**, which allows ATP generation through substrate-level phosphorylation.

Fill in the blank: The breakdown of glucose into pyruvate with ATP production is called _____.

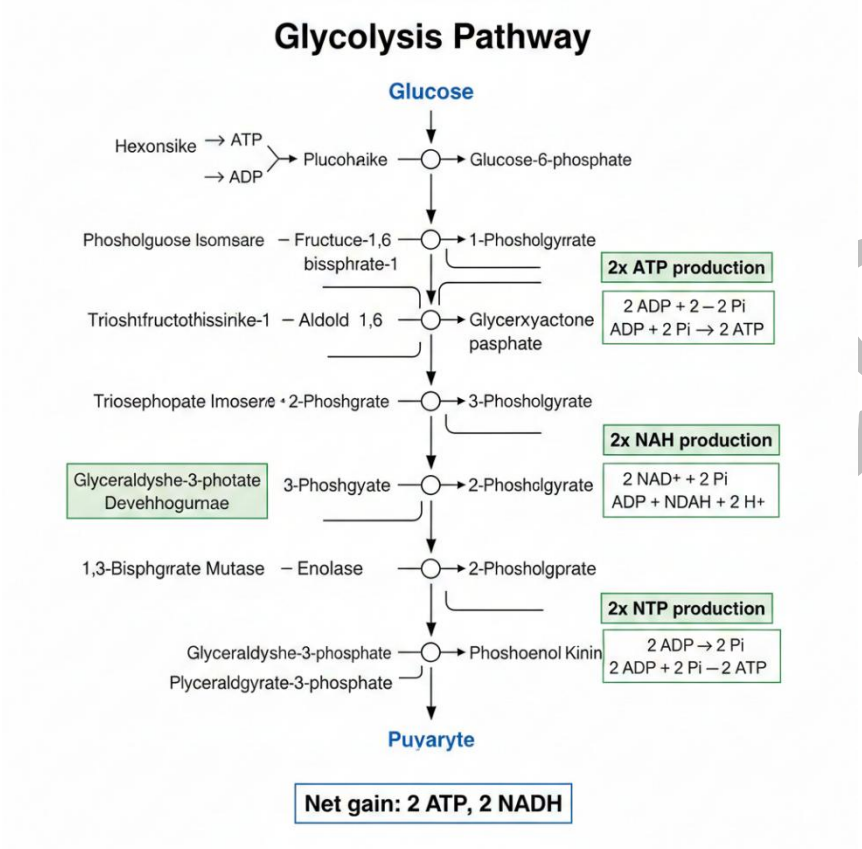


Figure 4.5 Overview of glycolysis in microbial metabolism.

4.3.2 Anabolic pathways

Anabolism refers to metabolic processes that build complex molecules from simpler ones, requiring energy input. Microbes use anabolic pathways to synthesise essential biomolecules such as amino acids, nucleotides, and lipids. These pathways are crucial for growth and reproduction, as they provide the structural and functional components of cells.

For example, amino acid biosynthesis involves the incorporation of nitrogen into carbon skeletons, while nucleotide biosynthesis provides the building blocks for DNA and RNA. Lipid biosynthesis contributes to membrane formation and energy storage.

Fill in the blank: The metabolic process that builds complex molecules from simpler ones is called _____.

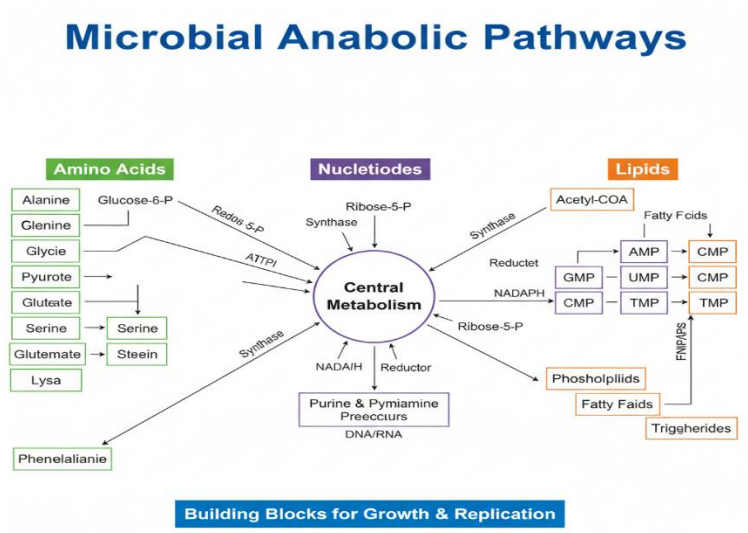


Figure 4.6 Examples of anabolic pathways in microbial physiology.

4.3.3 Amphibolic pathways

Amphibolic pathways are defined as metabolic routes that function in both catabolism and anabolism. The TCA cycle is a classic example, as it not only generates energy but also provides intermediates for biosynthesis. These dual-purpose pathways allow microbes to adapt flexibly to environmental conditions by balancing energy production with biosynthetic needs.

Fill in the blank: A pathway that functions in both catabolism and anabolism is called an _____ pathway.

Core Pathway	Catabolic Role	Anabolic Role (Biosynthesis)
TCA Cycle (Krebs Cycle)	Oxidizes Acetyl-CoA to produce CO_2, ATP (GTP), NADH, and $FADH_2$.	**α-ketoglutarate** and **Oxaloacetate** are used to synthesize amino acids (e.g., Glutamate, Aspartate).
Glycolysis	Breaks down Glucose into Pyruvate to generate ATP and NADH.	**Glucose-6-phosphate** can enter the Pentose Phosphate Pathway; **Dihydroxyacetone phosphate (DHAP)** is used for lipid synthesis.
Pentose Phosphate Pathway	Oxidizes Glucose-6-phosphate to produce NADPH for redox balance.	Provides **Ribose-5-phosphate** for nucleotide (DNA/RNA) synthesis and **Erythrose-4-phosphate** for aromatic amino acids.

Box 4.3 Examples of amphibolic pathways

4.3.4 Metabolic flux and control

Metabolic flux refers to the rate at which substrates and products flow through a metabolic pathway. Cells regulate flux to ensure that energy and biosynthetic demands are met efficiently. Control mechanisms include enzyme regulation, substrate availability, and feedback loops. Microbes often adjust metabolic flux in response to environmental changes, such as nutrient availability or oxygen levels. This regulation ensures survival and optimal growth under diverse conditions.

Fill in the blank: The rate at which substrates and products move through a pathway is called metabolic _____.

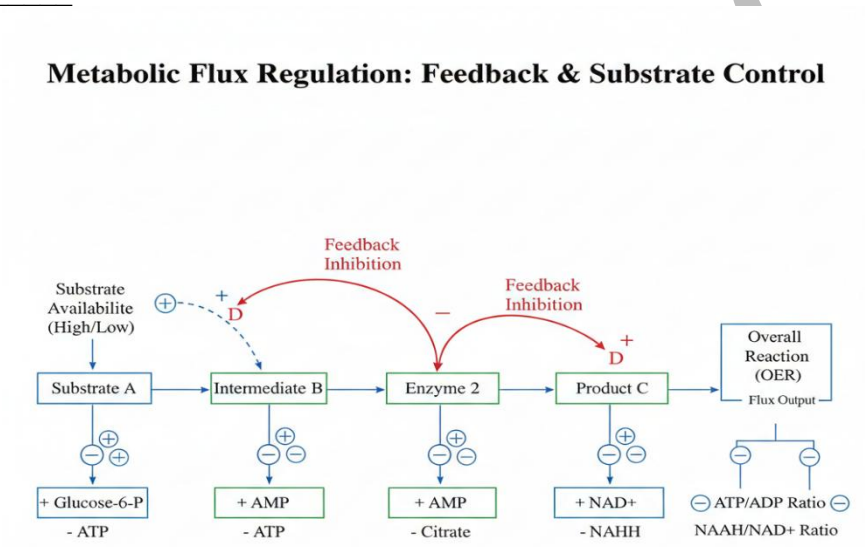


Figure 4.7 Regulation of metabolic flux in microbial pathways.

Pilot questions 4.3

1. What is catabolism and what is its primary function in microbes?
2. Name one major catabolic pathway that generates ATP.
3. Define anabolism and give one example of an anabolic product.
4. What is an amphibolic pathway?
5. What does metabolic flux refer to in microbial physiology?

4.4 Specialised Bioenergetic Processes in Microbes

4.4.1 Electron transport chains

Electron transport chains (ETCs) are defined as sequences of protein complexes and electron carriers that transfer electrons from donors to acceptors through redox reactions. This transfer is coupled to the movement of protons across membranes, creating an electrochemical gradient. In microbes, ETCs vary widely depending on the organism and its environment. Aerobic microbes typically use oxygen as the terminal electron acceptor, while anaerobic microbes may use nitrate, sulphate, or other compounds.

The diversity of microbial ETCs reflects their adaptability to different ecological niches. This flexibility allows microbes to exploit a wide range of energy sources.

Fill in the blank: In aerobic microbes, the terminal electron acceptor in the electron transport chain is _____.

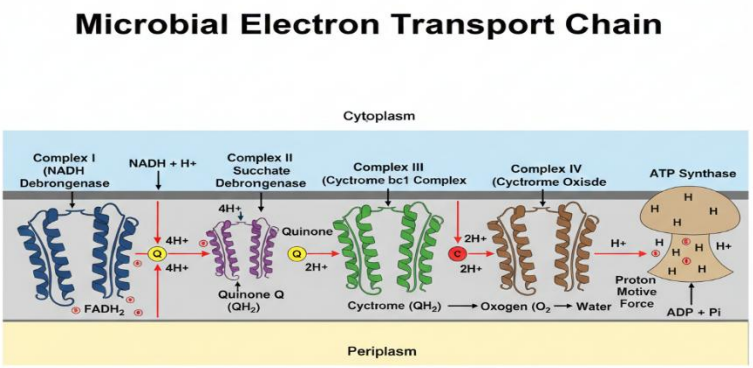


Figure 4.8 Electron transport chain in microbial metabolism.

4.4.2 Oxidative phosphorylation

Oxidative phosphorylation is defined as the process by which ATP is synthesised using energy derived from the electron transport chain. As electrons move through the ETC, protons are pumped across the membrane, generating a **proton motive force (PMF)**. This gradient drives protons back through **ATP synthase**, a molecular machine that couples proton flow to ATP production.

This process is highly efficient and is the primary means of ATP generation in aerobic microbes. The chemiosmotic theory proposed by Peter Mitchell explains how the PMF powers ATP synthesis.

Fill in the blank: The gradient that drives ATP synthesis during oxidative phosphorylation is called the proton _____ force.

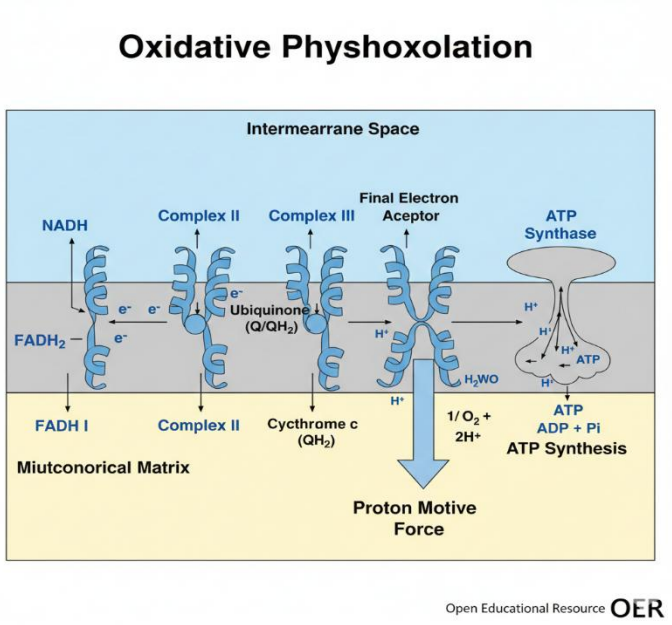


Figure 4.9 Oxidative phosphorylation and ATP synthesis in microbes.

4.4.3 Alternative energy sources

Microbes are remarkably versatile in their energy acquisition strategies. Some use **phototrophy**, defined as the process of capturing light energy to drive ATP synthesis. Others rely on **lithotrophy**, which involves the oxidation of inorganic compounds such as hydrogen, sulphur, or iron. In environments lacking oxygen, microbes may perform **anaerobic respiration**, using alternative electron acceptors like nitrate or sulphate.

These specialised processes enable microbes to colonise extreme environments, from deep-sea vents to nutrient-poor soils. Their metabolic diversity is a key factor in global biogeochemical cycles.

Fill in the blank: The process of using light energy to drive ATP synthesis is called _____.

Strategy	Energy Source	Electron Donor	Key Features
Phototrophy	Light (Photons)	H_2O, H_2S, or organic matter	Uses pigments (chlorophyll/bacteriochlorophyll) to convert light into chemical energy (ATP).
Lithotrophy	Inorganic Chemicals	H_2, S^0, Fe^{2+}, NH_3	Also known as "rock-eating." Electrons are extracted from inorganic molecules to fuel the ETC.

Strategy	Energy Source	Electron Donor	Key Features
Anaerobic Respiration	Organic/Inorganic Chemicals	Organic compounds or H_2	Uses an electron transport chain but replaces O_2 with alternative acceptors like NO_3^-, SO_4^{2-}, or CO_2.

Box 4.4 Alternative microbial energy sources

Pilot questions 4.4

1. What is the role of electron transport chains in microbial metabolism?
2. Name the terminal electron acceptor in aerobic respiration.
3. What is the proton motive force and how is it generated?
4. Which enzyme couples proton flow to ATP synthesis?
5. Give one example of an alternative microbial energy source.

Series Summary

This Series has explored the essential principles of bioenergetics, enzyme action, and metabolic pathways, all of which are central to microbial physiology. We began by examining the fundamentals of bioenergetics, including energy in biological systems, thermodynamic principles, and the role of ATP as the universal energy currency. We then moved on to enzyme structure and function, considering their properties, kinetics, and regulation. Following that, we studied metabolic pathways, focusing on catabolic, anabolic, and amphibolic processes, as well as the regulation of metabolic flux. Finally, we concluded with specialised bioenergetic processes in microbes, such as electron transport chains, oxidative phosphorylation, and alternative energy sources like phototrophy and lithotrophy.

By completing this Series, you should now be able to define and explain the principles of bioenergetics, analyse enzyme kinetics and regulation, describe the organisation of metabolic pathways, and evaluate specialised microbial energy processes. These abilities directly align with the Series Learning Outcomes, ensuring that you can apply knowledge of microbial metabolism to broader contexts in physiology and biochemistry.

Glossary of Terms

- **Activation energy**: The minimum amount of energy required to initiate a chemical reaction. Enzymes lower this energy, allowing reactions to occur more readily.
- **Allosteric regulation**: A form of enzyme control where molecules bind to a site other than the active site, changing the enzyme's activity.

- **Amphibolic pathway**: A metabolic route that functions in both catabolism and anabolism, such as the tricarboxylic acid (TCA) cycle.
- **Anabolism**: The set of metabolic processes that build complex molecules from simpler ones, requiring energy input.
- **ATP (adenosine triphosphate)**: The universal energy currency of the cell, storing energy in high-energy phosphate bonds.
- **Catabolism**: The set of metabolic processes that break down complex molecules into simpler ones, releasing energy.
- **Chemiosmotic theory**: The explanation of how the proton motive force generated by electron transport drives ATP synthesis.
- **Electron transport chain (ETC)**: A sequence of protein complexes and carriers that transfer electrons through redox reactions, coupled with proton pumping across membranes.
- **Enzyme**: A biological catalyst, usually a protein, that speeds up chemical reactions without being consumed.
- **Enzyme kinetics**: The study of the rates of enzyme-catalysed reactions, often described using the Michaelis-Menten model.
- **Feedback inhibition**: A regulatory mechanism where the end product of a pathway inhibits an enzyme earlier in the pathway.
- **Free energy (ΔG)**: The portion of energy available to do work in a system. A negative ΔG indicates a spontaneous reaction.
- **Glycolysis**: A catabolic pathway that breaks down glucose into pyruvate, producing ATP and NADH.
- **Lithotrophy**: The microbial process of obtaining energy by oxidising inorganic compounds such as hydrogen, sulphur, or iron.
- **Metabolic flux**: The rate at which substrates and products move through a metabolic pathway.
- **Oxidative phosphorylation**: The process of ATP synthesis powered by the proton motive force generated through the electron transport chain.
- **Phototrophy**: The microbial process of capturing light energy to drive ATP synthesis.
- **Proton motive force (PMF)**: The electrochemical gradient of protons across a membrane that drives ATP synthesis.
- **Thermodynamics**: The study of energy transformations, including the laws that govern conservation and entropy in biological systems.
- **Vmax**: The maximum rate of an enzyme-catalysed reaction when the enzyme is saturated with substrate.

Concept Check Questions 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

Objective questions

1. Which molecule is considered the universal energy currency of the cell? (Linked to SLO 4.3)

a. Glucose
b. ATP
c. NADH
d. Pyruvate

2. The substrate concentration at which the reaction rate is half of its maximum is called _____. (Linked to SLO 4.5)

a. Vmax
b. Km
c. ΔG
d. PMF

3. Which metabolic pathway functions in both catabolism and anabolism? (Linked to SLO 4.9)

a. Glycolysis
b. Fermentation
c. TCA cycle
d. Oxidative phosphorylation

4. In aerobic microbes, the terminal electron acceptor in the electron transport chain is _____. (Linked to SLO 4.11)

a. Nitrate
b. Oxygen
c. Sulphate
d. Carbon dioxide

5. What is the maximum rate of an enzyme-catalysed reaction called? (Linked to SLO 4.5)

a. Km
b. ΔG
c. Vmax
d. Flux

Short-answer questions

1. Define catabolism and give one example of a catabolic pathway. (Linked to SLO 4.7)
2. Explain the role of feedback inhibition in enzyme regulation. (Linked to SLO 4.6)
3. Describe the proton motive force and its role in oxidative phosphorylation. (Linked to SLO 4.12)
4. State the first law of thermodynamics and explain its relevance to microbial metabolism. (Linked to SLO 4.2)
5. What is metabolic flux and why is it important in microbial physiology? (Linked to SLO 4.10)

Long-answer questions

1. Analyse the role of ATP in energy coupling, providing examples of how exergonic and endergonic reactions are linked in microbial metabolism. (Linked to SLO 4.3)

2. Evaluate the diversity of microbial energy sources, comparing phototrophy, lithotrophy, and anaerobic respiration, and discuss their ecological significance. (Linked to SLO 4.13)

Answers to Concept Check Questions 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

Objective questions

1. b) ATP
2. b) Km
3. c) TCA cycle
4. b) Oxygen
5. c) Vmax

Short-answer questions

1. Catabolism is the breakdown of complex molecules into simpler ones, releasing energy. Example: glycolysis.
2. Feedback inhibition occurs when the end product of a pathway inhibits an enzyme earlier in the pathway, preventing overproduction.
3. The proton motive force is the electrochemical gradient of protons across a membrane. It drives protons through ATP synthase, powering ATP synthesis.
4. The first law of thermodynamics states that energy cannot be created or destroyed, only transformed. In microbes, it explains how energy is conserved during metabolism.
5. Metabolic flux is the rate at which substrates and products move through a pathway. It is important because it ensures energy and biosynthetic demands are balanced.

Long-answer questions

1. ***Basic response*:** ATP couples energy-releasing reactions, such as glucose breakdown, with energy-requiring reactions, such as biosynthesis. This ensures efficient energy use in microbial cells.***Value-added response*:** Beyond basic coupling, ATP also powers active transport, motility, and signal transduction. Its role in phosphorylation cascades highlights its importance in regulatory networks.
2. ***Basic response*:** Phototrophy uses light energy, lithotrophy uses inorganic compounds, and anaerobic respiration uses alternative electron acceptors. These processes allow microbes to survive in diverse environments. ***Value-added response*:** Ecologically, these strategies contribute to global cycles. Phototrophs drive primary production, lithotrophs recycle inorganic nutrients, and anaerobic microbes maintain ecosystem balance in oxygen-limited habitats.

Answers to Pilot Questions 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

Pilot Questions 4.1

1. Chemical energy.
2. Energy cannot be created or destroyed, only transformed.
3. A negative ΔG indicates a spontaneous reaction.
4. ATP.
5. Energy coupling is the use of energy released from exergonic reactions to drive endergonic reactions.

Pilot Questions 4.2

1. Enzymes are biological catalysts that speed up metabolic reactions.
2. It illustrates how enzyme specificity is achieved by the precise fit between substrate and active site.
3. Km represents the substrate concentration at which the reaction rate is half of its maximum.
4. Vmax.
5. Allosteric regulation and feedback inhibition.

Pilot Questions 4.3

1. Catabolism is the breakdown of complex molecules into simpler ones, releasing energy.
2. Glycolysis.
3. Anabolism is the building of complex molecules from simpler ones, requiring energy.
4. An amphibolic pathway functions in both catabolism and anabolism.
5. Metabolic flux refers to the rate at which substrates and products move through a pathway.

Pilot Questions 4.4

1. Electron transport chains transfer electrons and pump protons to generate an electrochemical gradient.
2. Oxygen.
3. The proton motive force is the gradient of protons across a membrane, generated by the ETC.
4. ATP synthase.
5. Phototrophy, lithotrophy, or anaerobic respiration.

References and Attributions 4: Bioenergetics, Enzyme Action, and Metabolic Pathways

General references

- Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock Biology of Microorganisms* (15th ed.). Pearson.
- Nelson, D. L., Cox, M. M. (2017). *Lehninger Principles of Biochemistry* (7th ed.). W. H. Freeman.

- OpenStax (2016). *Biology*. OpenStax CNX. Retrieved from https://openstax.org/books/biology

Illustration references and attributions

- *Figure 4.1 Forms of energy relevant to microbial physiology*

- Suggested AI prompt: “Generate a labelled diagram showing chemical, kinetic, and potential energy in microbial systems.”
- Search string: “forms of energy in microbial physiology diagram OER”

- *Box 4.1 Key thermodynamic principles in microbial metabolism*

- Suggested AI prompt: “Create a text box summarising the first and second laws of thermodynamics with microbial examples.”
- Search string: “thermodynamics in microbial metabolism summary OER”

- *Figure 4.2 Structure of ATP and its role in energy transfer*

- Suggested AI prompt: “Generate a labelled diagram of ATP showing phosphate groups and energy release during hydrolysis.”
- Search string: “ATP structure and energy coupling diagram OER”

- *Figure 4.3 Enzyme-substrate interaction in the lock-and-key model*

- Suggested AI prompt: “Generate a labelled diagram of enzyme-substrate interaction using the lock-and-key model.”
- Search string: “enzyme lock and key model diagram OER”

- *Figure 4.4 Michaelis-Menten curve showing Km and Vmax*

- Suggested AI prompt: “Generate a graph of Michaelis-Menten kinetics showing Km and Vmax.”
- Search string: “Michaelis-Menten kinetics graph OER”

- *Box 4.2 Mechanisms of enzyme regulation*

- Suggested AI prompt: “Create a text box summarising enzyme regulation mechanisms with examples.”
- Search string: “enzyme regulation mechanisms summary OER”

- *Figure 4.5 Overview of glycolysis in microbial metabolism*

- Suggested AI prompt: “Generate a labelled diagram of glycolysis showing glucose breakdown to pyruvate with ATP and NADH production.”
- Search string: “glycolysis pathway diagram OER”

- *Figure 4.6 Examples of anabolic pathways in microbial physiology*

- Suggested AI prompt: “Generate a labelled diagram showing anabolic pathways for amino acids, nucleotides, and lipids in microbes.”
- Search string: “anabolic pathways amino acids nucleotides lipids OER”

- *Box 4.3 Examples of amphibolic pathways*

- Suggested AI prompt: “Create a text box summarising amphibolic pathways with examples like the TCA cycle.”
- Search string: “amphibolic pathways examples OER”

- *Figure 4.7 Regulation of metabolic flux in microbial pathways*

- Suggested AI prompt: “Generate a diagram showing metabolic flux regulation with feedback inhibition and substrate availability.”
- Search string: “metabolic flux regulation diagram OER”

- *Figure 4.8 Electron transport chain in microbial metabolism*

- Suggested AI prompt: “Generate a labelled diagram of a microbial electron transport chain showing complexes, electron carriers, and proton pumping.”
- Search string: “microbial electron transport chain diagram OER”

- *Figure 4.9 Oxidative phosphorylation and ATP synthesis in microbes*

- Suggested AI prompt: “Generate a labelled diagram showing oxidative phosphorylation with proton motive force and ATP synthase.”
- Search string: “oxidative phosphorylation proton motive force diagram OER”

- *Box 4.4 Alternative microbial energy sources*

- Suggested AI prompt: “Create a text box summarising alternative microbial energy sources including phototrophy, lithotrophy, and anaerobic respiration.”
- Search string: “alternative microbial energy sources summary OER”

Course Code: MCB 424
Course Title: Microbial Physiology & Metabolism
Course Unit: 3 Units

Series 1: Bacterial Anatomy, Growth Dynamics, and Culture Systems
Series 2: Microbial Nutrition, Energy Metabolism, and Environmental Factors
Series 3: Biochemistry of Microbial Processes and Biosynthesis

Series 4: Bioenergetics, Enzyme Action, and Metabolic Pathways
Series 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Series 5: Laboratory Techniques and Assay Methods in Microbial Physiology
Series Outline

5.1 Principles of Microbial Laboratory Practice

- **5.1.1 Safety in the microbiology laboratory**: Sterile techniques, biosafety levels, and handling microbial cultures.
- **5.1.2 Equipment and instrumentation**: Microscopes, incubators, autoclaves, and spectrophotometers.
- **5.1.3 Culture handling and contamination control**: Aseptic transfer, culture storage, and contamination prevention.

5.2 Microbial Culture Techniques

- **5.2.1 Pure culture methods**: Streak plate, pour plate, and spread plate techniques.
- **5.2.2 Continuous and batch culture systems**: Chemostats, turbidostats, and closed systems.
- **5.2.3 Measurement of microbial growth**: Direct counts, viable counts, and turbidity measurements.

5.3 Biochemical and Physiological Assays

- **5.3.1 Enzyme assays**: Detection of enzyme activity, kinetics, and inhibition studies.
- **5.3.2 Metabolic profiling**: Carbohydrate utilisation, fermentation tests, and respiration assays.
- **5.3.3 Stress response assays**: Heat shock, oxidative stress, and pH tolerance tests.

5.4 Molecular and Analytical Techniques

- **5.4.1 Nucleic acid-based methods**: PCR, gel electrophoresis, and sequencing basics.
- **5.4.2 Protein analysis**: SDS-PAGE, Western blotting, and enzyme-linked assays.
- **5.4.3 Metabolite detection**: Chromatography and spectrophotometric methods.

5.5 Applied Microbial Assay Methods

- **5.5.1 Antibiotic susceptibility testing**: Disc diffusion, MIC determination, and resistance profiling.
- **5.5.2 Environmental microbiology assays**: Water quality testing, soil microbial activity, and bioremediation monitoring.
- **5.5.3 Industrial microbiology assays**: Fermentation monitoring, product yield analysis, and quality control.

Introduction

Laboratory techniques form the backbone of microbial physiology, as they provide the tools and methods needed to study microorganisms in detail. Without reliable laboratory practices, it would be impossible to investigate microbial growth, metabolism, or their responses to environmental changes. This Series will guide you through the essential techniques and assays used to explore microbial physiology, ensuring that you gain both theoretical understanding and practical competence.

The aim of this Series is to equip you with the knowledge and skills to apply laboratory methods in microbial physiology, from basic culture handling to advanced biochemical and molecular assays.

We shall commence this Series by examining the principles of microbial laboratory practice, including safety, equipment, and contamination control. Next, we will explore microbial culture techniques, focusing on pure culture methods, continuous and batch systems, and ways of measuring growth. Following that, we will move into biochemical and physiological assays, where you will learn how to assess enzyme activity, metabolic profiles, and stress responses. Afterwards, we will continue with molecular and analytical techniques such as PCR, protein analysis, and metabolite detection. Finally, we will wrap up by considering applied microbial assay methods, including antibiotic susceptibility testing, environmental assays, and industrial applications. This structured approach will provide you with a comprehensive toolkit for studying and applying microbial physiology in diverse contexts.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **5.1** demonstrate safe laboratory practices and explain the importance of biosafety in microbial research,
- **5.2** identify and operate essential microbiology laboratory equipment such as microscopes, incubators, and spectrophotometers,
- **5.3** apply aseptic techniques to handle microbial cultures and prevent contamination,
- **5.4** perform pure culture methods including streak plate, pour plate, and spread plate techniques,
- **5.5** differentiate between batch and continuous culture systems and analyse their applications,
- **5.6** measure microbial growth using direct counts, viable counts, and turbidity methods,
- **5.7** conduct enzyme assays to detect activity and evaluate kinetic parameters,
- **5.8** perform metabolic profiling through carbohydrate utilisation, fermentation, and respiration tests,
- **5.9** assess microbial stress responses using assays for heat shock, oxidative stress, and pH tolerance,
- **5.10** apply nucleic acid-based methods such as PCR and gel electrophoresis in microbial studies,
- **5.11** demonstrate protein analysis techniques including SDS-PAGE and Western blotting,
- **5.12** utilise metabolite detection methods such as chromatography and spectrophotometry,
- **5.13** carry out antibiotic susceptibility testing using disc diffusion and MIC determination,
- **5.14** evaluate microbial activity in environmental assays such as water quality and soil testing, and

5.15 apply microbial assay methods in industrial contexts including fermentation monitoring and product yield analysis.

5.1 Principles of Microbial Laboratory Practice

5.1.1 Safety in the microbiology laboratory

Laboratory safety refers to the set of practices and precautions designed to protect both the researcher and the environment when working with microorganisms. In microbiology, safety is paramount because microbes can pose risks ranging from mild contamination to serious infections. Key aspects include the use of **biosafety levels (BSLs)**, which are graded systems that classify laboratories according to the type of organisms handled and the precautions required. Basic safety practices involve wearing protective clothing, using aseptic techniques, and properly disposing of microbial waste. Sterile handling reduces the risk of contamination and ensures reliable experimental results.

Fill in the blank: The graded system that classifies laboratories according to the type of organisms handled is called _____ levels.

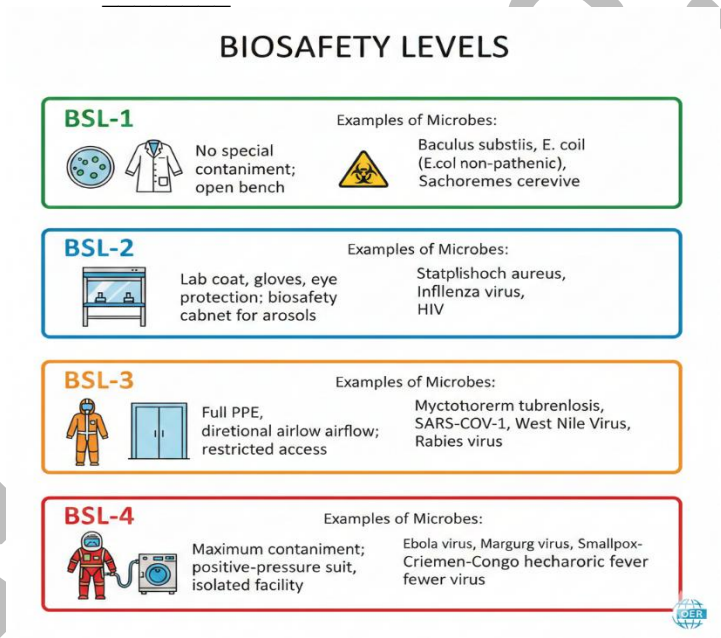


Figure 5.1 Biosafety levels in microbiology laboratories.

5.1.2 Equipment and instrumentation

Microbiology laboratories rely on specialised **equipment** to support microbial growth, observation, and analysis. Common instruments include the **microscope**, defined as an optical device used to magnify and observe microorganisms; the **incubator**, which maintains controlled temperature conditions for microbial growth; and the **autoclave**, which sterilises equipment and media using pressurised steam.

Another essential tool is the **spectrophotometer**, which measures the absorbance of light by microbial cultures and is often used to estimate cell density. Proper use and maintenance of these instruments are critical for accurate results.

Fill in the blank: The instrument used to sterilise equipment and media with pressurised steam is called an _____.

Plate 5.1 Essential equipment in a microbiology laboratory.

5.1.3 Culture handling and contamination control

Aseptic technique is defined as a set of procedures used to prevent contamination of cultures and the environment. It includes practices such as sterilising instruments before use, working near a flame or in a laminar flow hood, and avoiding unnecessary exposure of sterile materials. Proper culture handling ensures that microbial samples remain pure and experimental results are valid.

Contamination control also involves correct storage of cultures, such as refrigeration or cryopreservation, and careful labelling to avoid mix-ups. These practices are essential for reproducibility and reliability in microbial physiology experiments.

Fill in the blank: The set of procedures used to prevent contamination of cultures and the environment is called _____ technique.

Phase	Action	Purpose
Preparation	Clear the workspace and wipe down surfaces with **70% ethanol** or disinfectant.	Remove dust and decrease the microbial load on the bench.
2. Personal Safety	Wash hands thoroughly and wear appropriate PPE (lab coat, gloves, goggles).	Protect the researcher and prevent skin flora from entering the culture.

Phase	Action	Purpose
3. Sterilization	Light the Bunsen burner to create a sterile upward air current; flame loops or needles until **red hot**.	Kill any existing microbes on tools before they touch the sample.
4. Handling Caps	Never place tube caps on the bench. Hold them with your pinky finger.	Prevent environmental microbes on the desk from hitching a ride into the tube.
5. Flaming Necks	Pass the neck of glass culture tubes through the flame before and after pipetting.	Create convection currents that push air *out* of the tube, preventing contaminants from falling *in*.
6. Plate Work	Open Petri dish lids at a **45-degree angle** (clam-shelling) only when necessary.	Minimize the surface area exposed to falling airborne particles.
7. Final Clean up	Re-sterilize loops, turn off the burner, and disinfect the bench again.	Ensure the workspace is safe for the next user and prevent cross-contamination.

Box 5.1 Key steps in aseptic technique

Pilot questions 5.1

What is the purpose of biosafety levels in microbiology laboratories?
Name two pieces of equipment commonly used in microbial laboratories.
Which instrument is used to sterilise media and equipment with steam?
Define aseptic technique.
Why is contamination control important in microbial physiology experiments?

5.2 Microbial Culture Techniques

5.2.1 Pure culture methods

Pure culture methods are techniques used to isolate a single type of microorganism from a mixed population. This is essential for studying microbial physiology, as mixed cultures can lead to misleading results. Common methods include the **streak plate technique**, where a loop is used to spread microbes across agar to obtain isolated colonies; the **pour plate technique**, where diluted samples are mixed with molten agar and poured into plates; and the **spread plate technique**, where diluted samples are spread evenly across the surface of solid agar.
These methods ensure that researchers can obtain pure colonies for further study, such as biochemical assays or genetic analysis.

Fill in the blank: The technique where microbes are spread across agar to obtain isolated colonies is called the _____ plate method.

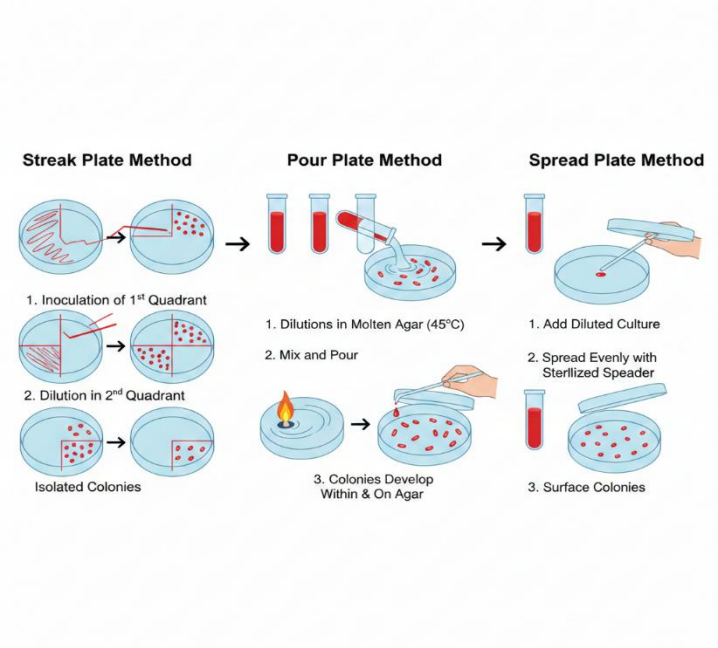


Figure 5.2 Common pure culture methods in microbiology.

5.2.2 Continuous and batch culture systems

Microbes can be grown in different culture systems depending on the purpose of the experiment. **Batch culture** is defined as a closed system where microbes are grown in a fixed volume of medium without additional nutrient input. Growth follows a predictable pattern: lag phase, exponential phase, stationary phase, and death phase.

In contrast, **continuous culture** is an open system where fresh medium is continuously supplied, and waste products are removed. Systems such as the **chemostat** and **turbidostat** maintain microbial populations in a steady state, allowing long-term studies of microbial physiology.

Fill in the blank: A culture system where fresh medium is continuously supplied and waste removed is called a _____ culture.

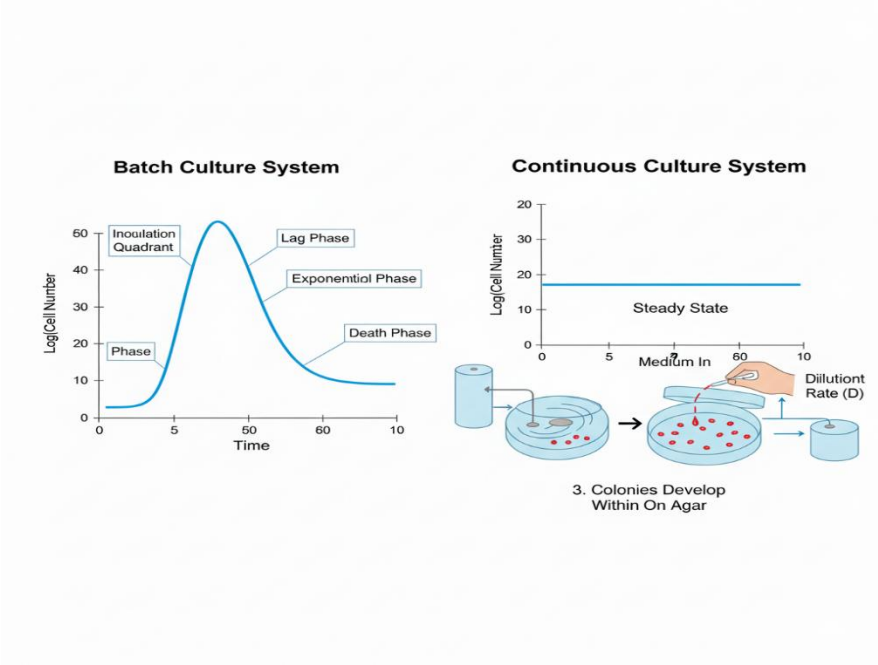


Figure 5.3 Batch and continuous culture systems.

5.2.3 Measurement of microbial growth

Accurate measurement of microbial growth is vital for understanding physiology and metabolism. Methods include **direct counts**, where cells are counted under a microscope or using electronic counters; **viable counts**, which estimate living cells by counting colonies formed on agar plates; and **turbidity measurements**, where a spectrophotometer measures the cloudiness of a culture as an indirect estimate of cell density.

Each method has advantages and limitations. Direct counts provide immediate results but cannot distinguish live from dead cells. Viable counts measure only living cells but require incubation time. Turbidity is quick and convenient but only provides relative estimates.

Fill in the blank: The method that measures microbial growth by assessing the cloudiness of a culture is called _____ measurement.

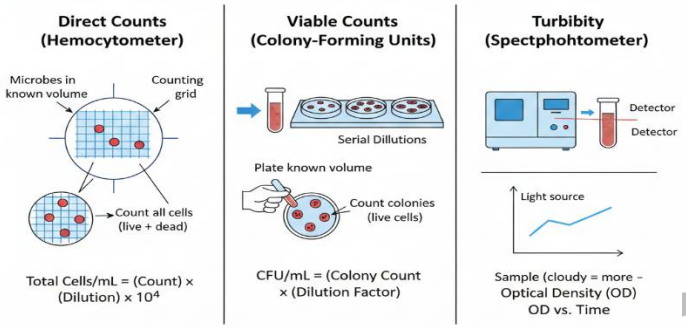


Figure 5.4 Methods of measuring microbial growth.

Pilot questions 5.2

What is the purpose of pure culture methods in microbiology?
Name one technique used to isolate pure cultures.
Define batch culture and describe its growth phases.
What is a chemostat used for?
List three methods of measuring microbial growth.

5.3 Biochemical and Physiological Assays

5.3.1 Enzyme assays

Enzyme assays are experimental procedures used to detect and measure the activity of enzymes. They provide information about how enzymes catalyse reactions, their efficiency, and how they respond to inhibitors. A common approach is to monitor the conversion of a substrate into a product, often using spectrophotometric methods to measure changes in absorbance.
Enzyme assays can be used to determine kinetic parameters such as **Km** (substrate concentration at half-maximal velocity) and **Vmax** (maximum reaction rate). These values help in understanding enzyme efficiency and regulation.
Fill in the blank: The maximum rate of an enzyme-catalysed reaction is referred to as _____.

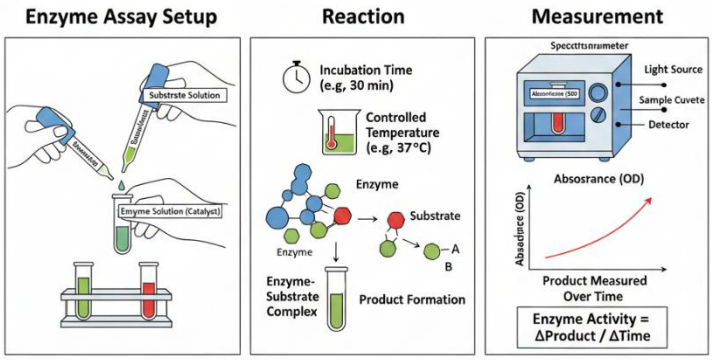


Figure 5.5 Basic setup of an enzyme assay.

5.3.2 Metabolic profiling

Metabolic profiling refers to the analysis of microbial metabolic activities, often by testing the ability of microbes to utilise different substrates. Common methods include **carbohydrate utilisation tests**, which assess whether microbes can ferment sugars, and **respiration assays**, which measure oxygen consumption or production of metabolic by-products.

Fermentation tests often use pH indicators to detect acid production, while respiration assays may involve monitoring gas exchange. These tests provide insights into microbial physiology and can be used for identification purposes.

Fill in the blank: The analysis of microbial metabolic activities, such as carbohydrate utilisation, is called metabolic _____.

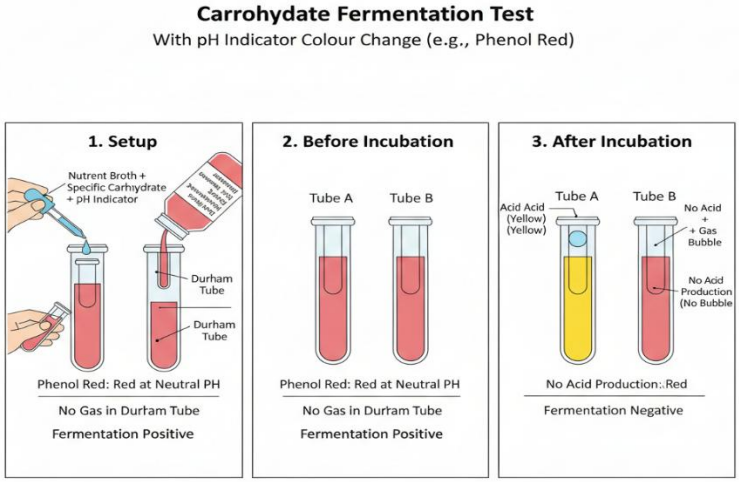


Figure 5.6 Carbohydrate fermentation test in metabolic profiling.

5.3.3 Stress response assays

Stress response assays are methods used to evaluate how microbes cope with environmental stressors such as heat, oxidative agents, or changes in pH. For example, **heat shock assays** expose microbes to elevated temperatures to test survival, while **oxidative stress assays** measure tolerance to reactive oxygen species. **pH tolerance tests** assess microbial growth under acidic or alkaline conditions.

These assays are important for understanding microbial adaptation and resilience, which can be critical in industrial and clinical contexts.

Fill in the blank: Assays that evaluate microbial tolerance to environmental changes are called stress _____ assays.

Stress Type	Common Assay Method	Physiological Response Measured
Heat Shock	**Incubation at supra-optimal temperatures** (e.g., shifting 37°C to 42°C) followed by viable plate counts.	Expression of **Heat Shock Proteins (HSPs)** like DnaK/GroEL that refold denatured proteins.
Oxidative Stress	**Disk Diffusion or MIC** using hydrogen peroxide ($H_{2}O_{2}$) or paraquat.	Induction of **Catalase or Superoxide Dismutase**

Stress Type	Common Assay Method	Physiological Response Measured
		(SOD) to neutralize reactive oxygen species (ROS).
pH Tolerance	**Growth curve analysis** in buffered media ranging from acidic (pH 3) to alkaline (pH 9).	Activation of **amino acid decarboxylases** (to raise pH) or **proton pumps** (to maintain homeostasis).
Osmotic Stress	**Turbidity measurements** in media with increasing concentrations of NaCl or sucrose.	Accumulation of **compatible solutes** (e.g., proline, betaine) to prevent plasmolysis.

Box 5.2 Examples of microbial stress response assays

Pilot questions 5.3

What is the purpose of enzyme assays in microbial physiology?
Define Vmax in the context of enzyme kinetics.
What does metabolic profiling reveal about microbes?
Name one method used to test microbial fermentation.
Give two examples of stress response assays.

5.4 Molecular and Analytical Techniques

5.4.1 Nucleic acid-based methods

Nucleic acid-based methods are laboratory techniques used to study DNA and RNA in microbes. One of the most widely used is **polymerase chain reaction (PCR)**, which amplifies specific DNA sequences, making them easier to analyse. Another important technique is **gel electrophoresis**, defined as a method that separates nucleic acids by size when an electric current is applied. Sequencing methods, though more advanced, allow researchers to determine the exact order of nucleotides in microbial genomes.

These methods are essential for identifying microbes, studying genetic variation, and monitoring gene expression.

Fill in the blank: The technique that separates nucleic acids by size using an electric current is called gel _____.

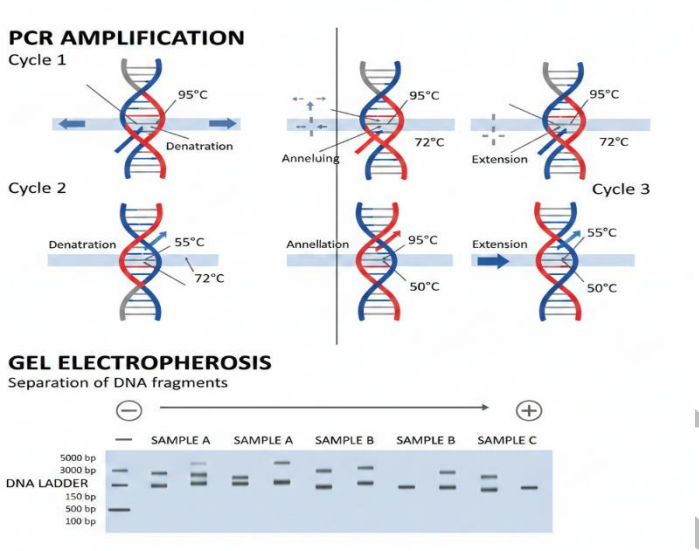


Figure 5.7 Nucleic acid-based methods in microbial physiology.

5.4.2 Protein analysis

Protein analysis refers to techniques used to study microbial proteins, their structure, and function. A common method is **SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis)**, which separates proteins based on size. Another is the **Western blot**, which transfers proteins from a gel to a membrane and uses antibodies to detect specific proteins. These methods are crucial for understanding microbial physiology, as proteins carry out most cellular functions, including enzyme activity and structural roles.

Fill in the blank: The technique that separates proteins based on size using a gel is called _____-PAGE.

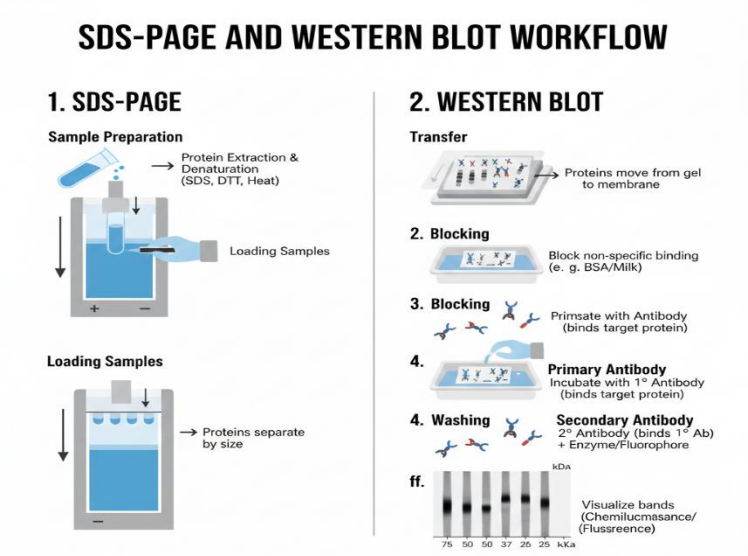


Figure 5.8 Protein analysis methods in microbiology.

5.4.3 Metabolite detection

Metabolite detection involves identifying and quantifying small molecules produced during microbial metabolism. Techniques include **chromatography**, defined as a method that separates compounds based on their chemical properties, and **spectrophotometry**, which measures the absorbance of light by metabolites.

These methods help researchers understand metabolic pathways, detect by-products, and monitor microbial activity in different environments.

Fill in the blank: The method that separates compounds based on chemical properties is called _____.

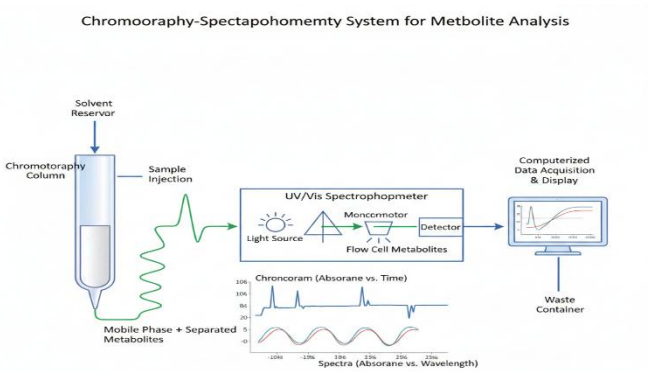


Figure 5.9 Metabolite detection methods in microbial physiology.

Pilot questions 5.4

What is the purpose of PCR in microbial studies?
Define gel electrophoresis.
Which technique separates proteins based on size?
What is the role of antibodies in Western blotting?
Name two methods used for metabolite detection.

5.5 Applied Microbial Assay Methods

5.5.1 Antibiotic susceptibility testing

Antibiotic susceptibility testing is a set of methods used to determine how sensitive or resistant microbes are to specific antibiotics. One common method is the **disc diffusion test**, where paper discs impregnated with antibiotics are placed on an agar plate inoculated with bacteria. Zones of inhibition around the discs indicate sensitivity. Another method is the **minimum inhibitory concentration (MIC) determination**, which identifies the lowest concentration of an antibiotic that prevents visible microbial growth.

These tests are crucial in clinical microbiology for guiding treatment decisions and monitoring the spread of antibiotic resistance.

Fill in the blank: The lowest concentration of an antibiotic that prevents visible microbial growth is called the _____.

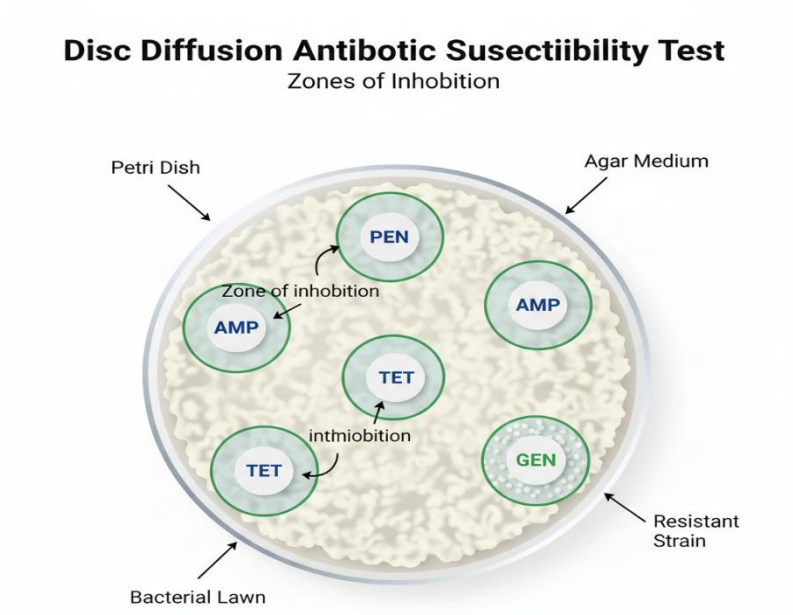


Figure 5.10 Antibiotic susceptibility testing using disc diffusion.

5.5.2 Environmental microbiology assays

Environmental microbiology assays are methods used to assess microbial activity in natural settings such as water, soil, and air. For example, **water quality testing** often involves detecting coliform bacteria as indicators of contamination. **Soil microbial activity assays** measure processes such as nitrogen fixation or organic matter decomposition. These assays help monitor ecosystem health and detect pollution.

Microbes play vital roles in biogeochemical cycles, and environmental assays provide insights into their contributions and resilience in changing environments.

Fill in the blank: The presence of coliform bacteria in water is often used as an indicator of _____ contamination.

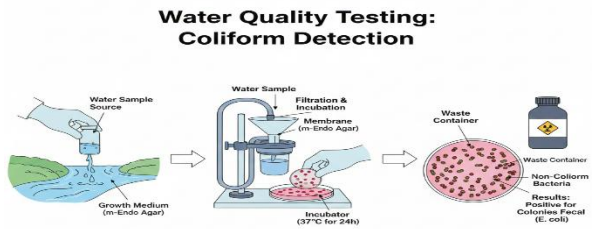


Figure 5.11 Environmental microbiology assays for water quality.

5.5.3 Industrial microbiology assays

Industrial microbiology assays are techniques used to monitor and optimise microbial processes in industrial applications. Examples include **fermentation monitoring**, where microbial growth and product yield are tracked, and **quality control assays**, which ensure that microbial products such as antibiotics, enzymes, or fermented foods meet required standards. These assays are essential for industries that rely on microbial activity, including pharmaceuticals, food production, and biotechnology. They ensure efficiency, safety, and consistency in microbial-based processes.

Fill in the blank: Monitoring microbial growth and product yield in industrial processes is called _____ monitoring.

Category	Primary Objective	Common Methods/Assays
Fermentation Monitoring	To track real-time microbial growth and metabolic state during the "run."	**Biomass measurement** (Optical Density/Dry weight), **pH/Dissolved Oxygen** tracking, and **Substrate consumption** (e.g., glucose depletion).
Product Yield Analysis	To quantify the amount and purity of the target metabolite produced.	**Chromatography** (HPLC, GC) for titer quantification, **Spectrophotometry**, and **Electrophoresis** for protein characterization.
Quality Control (QC)	To ensure the final product is safe, potent, and free of contaminants.	**Sterility testing**, **Endotoxin assays** (LAL test), **Bioburden analysis**, and **Genetic stability** checks of the production strain.

Box 5.3 Examples of industrial microbiology assays

Pilot questions 5.5

What is the purpose of antibiotic susceptibility testing?
Define MIC in the context of antibiotic testing.
Name one indicator organism used in water quality testing.
What microbial process is commonly measured in soil assays?
Give two examples of industrial microbiology assays.

Series Summary

This Series has focused on the essential laboratory techniques and assay methods that underpin microbial physiology. We began with the principles of microbial laboratory practice, highlighting safety, equipment, and aseptic handling. We then moved on to microbial culture

techniques, exploring pure culture methods, batch and continuous systems, and approaches to measuring microbial growth. Following that, we examined biochemical and physiological assays, including enzyme activity, metabolic profiling, and stress response tests. We continued with molecular and analytical techniques such as PCR, protein analysis, and metabolite detection. Finally, we concluded with applied microbial assay methods, covering antibiotic susceptibility testing, environmental assays, and industrial applications.
By completing this Series, you should now be able to demonstrate safe laboratory practices, apply culture techniques, conduct biochemical and physiological assays, utilise molecular and analytical methods, and evaluate applied microbial assays in clinical, environmental, and industrial contexts. These abilities directly align with the Series Learning Outcomes, ensuring that you are equipped with both the theoretical knowledge and practical skills necessary to investigate and apply microbial physiology effectively.

Glossary of Terms

Antibiotic susceptibility testing: Methods used to determine how sensitive or resistant microbes are to antibiotics, such as disc diffusion and MIC determination.
Aseptic technique: A set of procedures used to prevent contamination of cultures and the environment during laboratory work.
Autoclave: Equipment that sterilises laboratory materials using pressurised steam.
Batch culture: A closed system of microbial growth where nutrients are not replenished and waste accumulates, leading to distinct growth phases.
Biosafety levels (BSLs): Graded classifications of laboratories based on the type of organisms handled and the safety precautions required.
Chemostat: A continuous culture system where fresh medium is supplied and waste removed, maintaining microbes in steady-state growth.
Disc diffusion test: An antibiotic susceptibility method where paper discs containing antibiotics are placed on agar to observe zones of inhibition.
Enzyme assay: An experimental procedure used to detect and measure enzyme activity by monitoring substrate conversion into products.
Gel electrophoresis: A technique that separates nucleic acids or proteins by size using an electric current.
Incubator: Laboratory equipment that maintains controlled temperature conditions for microbial growth.
Metabolic profiling: Analysis of microbial metabolic activities, such as carbohydrate utilisation, fermentation, and respiration.
Metabolite detection: Methods used to identify and quantify small molecules produced during microbial metabolism, often using chromatography or spectrophotometry.
Minimum inhibitory concentration (MIC): The lowest concentration of an antibiotic that prevents visible microbial growth.
PCR (polymerase chain reaction): A technique used to amplify specific DNA sequences for easier analysis.
Pure culture methods: Techniques such as streak plate, pour plate, and spread plate used to isolate a single type of microorganism.
SDS-PAGE: A protein analysis method that separates proteins based on size using polyacrylamide gel electrophoresis.

Spectrophotometer: An instrument that measures the absorbance of light by microbial cultures, often used to estimate cell density.
Stress response assays: Tests that evaluate microbial tolerance to environmental stressors such as heat, oxidative agents, or pH changes.
Turbidostat: A continuous culture system that maintains microbial populations by adjusting nutrient supply based on turbidity measurements.
Viable count: A method of measuring microbial growth by counting colonies formed on agar plates, representing living cells only.

Concept Check Questions 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Objective questions

Which piece of equipment sterilises laboratory materials using pressurised steam? (Linked to SLO 5.2)

Incubator
Autoclave
Spectrophotometer
Microscope

The technique used to isolate a single type of microorganism from a mixed population is called _____. (Linked to SLO 5.4)

Batch culture
Pure culture method
Continuous culture
Stress assay

Which method separates proteins based on size using polyacrylamide gel? (Linked to SLO 5.11)

PCR
SDS-PAGE
Western blot
Gel electrophoresis

The lowest concentration of an antibiotic that prevents visible microbial growth is known as _____. (Linked to SLO 5.13)

MIC
Vmax
Km
Zone of inhibition

Which culture system maintains microbial populations in steady-state growth by continuously supplying nutrients? (Linked to SLO 5.5)

Batch culture
Chemostat
Turbidostat
Pour plate

Short-answer questions

Define aseptic technique and explain its importance in microbial laboratory practice. (Linked to SLO 5.3)
Describe one method used to measure microbial growth and state its limitation. (Linked to SLO 5.6)
Explain the principle of PCR and its application in microbial studies. (Linked to SLO 5.10)
What is the purpose of metabolic profiling in microbial physiology? (Linked to SLO 5.8)
Give two examples of stress response assays and explain what they measure. (Linked to SLO 5.9)

Long-answer questions

Analyse the role of enzyme assays in microbial physiology, highlighting how kinetic parameters such as Km and Vmax are determined and interpreted. (Linked to SLO 5.7)
Evaluate the significance of applied microbial assay methods in clinical, environmental, and industrial contexts, providing examples of their practical applications. (Linked to SLO 5.13, SLO 5.14, SLO 5.15)

Answers to Concept Check Questions 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Objective questions

b) Autoclave
b) Pure culture method
b) SDS-PAGE
a) MIC
b) Chemostat

Short-answer questions

Aseptic technique is a set of procedures used to prevent contamination of cultures and the environment. It is important because it ensures experimental reliability and safety.
Turbidity measurement uses a spectrophotometer to estimate cell density by measuring cloudiness. Its limitation is that it cannot distinguish live from dead cells.
PCR amplifies specific DNA sequences using repeated cycles of heating and cooling. It is used to identify microbes, study genetic variation, and monitor gene expression.
Metabolic profiling reveals the ability of microbes to utilise different substrates, providing insights into physiology and aiding microbial identification.

Heat shock assays measure survival at elevated temperatures, while oxidative stress assays assess tolerance to reactive oxygen species.

Long-answer questions

***Basic response*:** Enzyme assays measure enzyme activity by monitoring substrate conversion into products. Km and Vmax are determined from reaction rates at varying substrate concentrations, providing insights into enzyme efficiency. ***Value-added response*:** Beyond basic kinetics, enzyme assays can reveal regulatory mechanisms, inhibitor effects, and physiological adaptations. They are also applied in drug development and industrial enzyme optimisation.

***Basic response*:** Applied microbial assays include antibiotic susceptibility testing in clinical settings, water quality testing in environmental microbiology, and fermentation monitoring in industry. These methods ensure safety and efficiency. ***Value-added response*:** Their broader significance lies in combating antibiotic resistance, maintaining ecosystem health, and driving biotechnological innovation. For example, MIC determination informs treatment strategies, coliform detection safeguards public health, and industrial assays optimise large-scale microbial production.

Answers to Pilot Questions 5: Laboratory Techniques and Assay Methods in Microbial Physiology

Answers to Pilot Questions 5.1

To classify laboratories according to the organisms handled and ensure appropriate safety measures.
Microscope and autoclave.
Autoclave.
Aseptic technique is a set of procedures used to prevent contamination of cultures and the environment.
It ensures experimental reliability and prevents misleading results.

Answers to Pilot Questions 5.2

To isolate a single type of microorganism for study.
Streak plate technique.
A closed system where microbes grow in fixed medium, showing lag, exponential, stationary, and death phases.
To maintain continuous microbial growth in steady state.
Direct counts, viable counts, and turbidity measurements.

Answers to Pilot Questions 5.3

To detect and measure enzyme activity in microbes.
Vmax is the maximum rate of an enzyme-catalysed reaction.
It reveals microbial metabolic activities and substrate utilisation.

Carbohydrate fermentation test.
Heat shock assay and oxidative stress assay.

Answers to Pilot Questions 5.4

To amplify specific DNA sequences for easier analysis.
A method that separates nucleic acids by size using an electric current.
SDS-PAGE.
To detect specific proteins using antibodies.
Chromatography and spectrophotometry.

Answers to Pilot Questions 5.5

To determine microbial sensitivity or resistance to antibiotics.
Minimum inhibitory concentration (MIC).
Coliform bacteria.
Nitrogen fixation.
Fermentation monitoring and quality control assays.

References and Attributions 5: Laboratory Techniques and Assay Methods in Microbial Physiology

General references

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Nelson, D. L., & Cox, M. M. (2017). *Lehninger Principles of Biochemistry* (7th ed.). W. H. Freeman.

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Illustration references and attributions

Figure 5.1 Biosafety levels in microbiology laboratories

Suggested AI prompt: “Generate a labelled diagram showing biosafety levels 1 to 4 with examples of microbes handled at each level.”
Search string: “biosafety levels microbiology diagram OER”

Plate 5.1 Essential equipment in a microbiology laboratory

Suggested AI prompt: “Generate an image showing microscope, incubator, autoclave, and spectrophotometer in a microbiology lab setting.”
Search string: “microbiology laboratory equipment images OER”

Box 5.1 Key steps in aseptic technique

Suggested AI prompt: “Create a text box summarising aseptic technique steps in microbiology.”

Search string: “aseptic technique microbiology summary OER”

Figure 5.2 Common pure culture methods in microbiology

Suggested AI prompt: “Generate a labelled diagram showing streak plate, pour plate, and spread plate techniques for isolating pure cultures.”
Search string: “pure culture methods streak pour spread plate diagram OER”

Figure 5.3 Batch and continuous culture systems

Suggested AI prompt: “Generate a diagram comparing microbial growth in batch culture versus continuous culture systems.”
Search string: “batch vs continuous culture microbial growth diagram OER”

Figure 5.4 Methods of measuring microbial growth

Suggested AI prompt: “Generate a diagram showing direct counts, viable counts, and turbidity methods for measuring microbial growth.”
Search string: “methods of measuring microbial growth diagram OER”

Figure 5.5 Basic setup of an enzyme assay

Suggested AI prompt: “Generate a labelled diagram showing enzyme assay setup with substrate, enzyme, and product measurement.”
Search string: “enzyme assay setup diagram OER”

Figure 5.6 Carbohydrate fermentation test in metabolic profiling

Suggested AI prompt: “Generate a labelled diagram showing carbohydrate fermentation test with pH indicator colour change.”
Search string: “carbohydrate fermentation test diagram OER”

Box 5.2 Examples of microbial stress response assays

Suggested AI prompt: “Create a text box summarising microbial stress response assays including heat shock, oxidative stress, and pH tolerance.”
Search string: “microbial stress response assays summary OER”

Figure 5.7 Nucleic acid-based methods in microbial physiology

Suggested AI prompt: “Generate a labelled diagram showing PCR amplification and gel electrophoresis separation of DNA fragments.”
Search string: “PCR and gel electrophoresis diagram OER”

Figure 5.8 Protein analysis methods in microbiology

Suggested AI prompt: “Generate a labelled diagram showing SDS-PAGE and Western blot workflow for protein analysis.”
Search string: “SDS-PAGE and Western blot diagram OER”

Figure 5.9 Metabolite detection methods in microbial physiology

Suggested AI prompt: “Generate a labelled diagram showing chromatography setup and spectrophotometric detection of metabolites.”
Search string: “chromatography and spectrophotometry diagram OER”

Figure 5.10 Antibiotic susceptibility testing using disc diffusion

Suggested AI prompt: “Generate a labelled diagram showing disc diffusion antibiotic susceptibility test with zones of inhibition.”
Search string: “antibiotic susceptibility testing disc diffusion diagram OER”

Figure 5.11 Environmental microbiology assays for water quality

Suggested AI prompt: “Generate a labelled diagram showing water quality testing with coliform detection.”
Search string: “water quality testing coliform bacteria diagram OER”

Box 5.3 Examples of industrial microbiology assays

Suggested AI prompt: “Create a text box summarising industrial microbiology assays including fermentation monitoring, product yield analysis, and quality control.”
Search string: “industrial microbiology assays summary OER”