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كلية العلوم و التكنولوجيا
قسم هندسة الطرائق

Course handout

Environmental Microbiology and Biochemistry

For First year of the Master's degree in Environmental Process Engineering

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Foreword

*This teaching handout on « **environmental microbiology and biochemistry** » is intended for first-year Master's students in environmental process engineering under the LMD system. It is designed as an educational resource intended to facilitate learning, support the structured acquisition of knowledge and serve as a guide for students.*

*Its design and structure are the result of several years of teaching experience. As such, it serves as a reference document compiling all the essential knowledge related to the subject of « **Environmental Microbiology and Biochemistry** ».*

The course content presents the fundamental concepts of the subject and is illustrated with numerous diagrams and schematic representations.

The text is divided into two major parts:

-The first part focuses on environmental microbiology: covering the morphology and functional anatomy of bacteria, the biodiversity of microorganisms and their environment (soil, air, and water), as well as their role in bio-elements' cycles.

-The second part focuses on environmental biochemistry, addressing the molecular components of the cell, such as proteins, lipids and carbohydrates, and the role of microorganisms in their enzymatic breakdown.

Abstract

Environmental microbiology describes the relationship between microorganisms and their environment.

Microorganisms include bacteria, fungi, algae, and yeast. Each category is characterized by a classification based on several parameters, such as morphology and anatomy. Bacterial morphology refers to the shape of bacterial cells, their dimensions, and their arrangements. As for anatomy, it illustrates the various organelles that make up a bacterium, such as the flagella, the capsule, the cell wall, the cytoplasmic membrane, and the cytoplasm. Thus, each component plays a role in the life cycle of bacteria. The bacterial cell is composed of organic and inorganic matter, represented by the formula $C_5H_7O_2N$. Microorganisms need basic elements such as water, nitrogen, sulfur, phosphorus, minerals, and specific elements for their growth. Their study is based on sampling and microscopic observation.

Microorganisms are found in all ecosystems: in the soil, in aquatic environments, in the air, and in wastewater. They play a very important role in the environment, such as in the nitrogen (N), carbon (C), oxygen (O), sulfur (S), and phosphorus (P) cycles. Bacteria have the ability to break down proteins into amino acids, carbohydrates into sugars, and lipids into fatty acids (CH_3-$(CH_2)_n$-COOH). Cellulosic waste and hydrocarbons are also biodegraded by specific strains of bacteria and fungi. For each compound, biodegradation occurs in several stages. The degradation process is known as enzymology, characterized by enzyme kinetics (the Michaelis-Menten hypothesis), which studies the rate of chemical or biochemical reactions in which the enzyme (E) converts the substrate (S) into a product (P) without altering the equilibrium state.

Keywords: *Environmental microbiology, Microorganisms, Environmental biochemistry, Biodegradation, Enzymology.*

Résumé

Résumé

La microbiologie de l'environnement décrit la relation qui existe entre les micro-organismes et leur environnement. Les micro-organismes regroupent les bactéries, les champignons, les algues et les levures. Chaque catégorie est caractérisée par une classification en se basant sur plusieurs paramètres comme la morphologie et l'anatomie. La morphologie des bactéries porte sur la forme des cellules bactériennes, leurs dimensions et leurs arrangements. Quant à l'anatomie, elle illustre les différents organites qui constituent une bactérie comme les flagelles, la capsule, la paroi, la membrane cytoplasmique, le cytoplasme. Ainsi chaque constituant joue un rôle dans le cycle de vie des bactéries. La cellule bactérienne est constituée de matière organique et inorganique représentée par la formule $C_5H_7O_2N$. Les micro-organismes ont besoin des éléments élémentaires comme l'eau, l'azote, le soufre, le phosphore, les éléments minéraux et les éléments spécifiques pour leur croissance. Leur étude est basée sur l'échantillonnage et l'observation aux microscopes.

Les micro-organismes se trouvent dans tous les écosystèmes : le sol, dans les milieux aquatiques, dans l'air et dans les eaux usées. Ils jouent un rôle très important dans l'environnement tels que le cycle d'azote (N), de carbone ©, d'oxygène (O), du soufre (S) et du phosphore (P). Les bactéries ont la capacité de dégrader les protéines en acides aminés, les glucides en oses et les lipides en acides gras (CH_3-(CH_2) n-COOH). Les déchets cellulosiques et les hydrocarbures sont aussi biodégradés par des souches de bactéries et champignons spécifiques. Pour chaque composé, la biodégradation passe par plusieurs étapes. Le processus de dégradation est connu sous le nom d'enzymologie caractérisé par la cinétique enzymatique, (hypothèse de Mickaelis-Menten), qui étudie la vitesse des réactions chimiques ou biochimiques, où l'enzyme (E) transforme le substrat (S) en produit (P) sans modifier l'état d'équilibre.

Les mots-clés : *La microbiologie de l'environnement, Les micro-organismes, La biochimie de l'environnement, La biodégradation, L'enzymologie.*

الملخص

يصف علم الأحياء الدقيقة البيئية العلاقة القائمة والوطيدة بين الكائنات الحية الدقيقة وبيئتها. وتشمل الكائنات الدقيقة البكتيريا والفطريات والطحالب والخميرة والكائنات الأولية.

تتميز كل فئة بتصنيف يستند إلى عدة معايير مثل الشكل والتشريح. ويتناول شكل البكتيريا، شكل الخلايا البكتيرية وأبعادها وترتيبها.

أما علم التشريح، فيوضح المكونات الخلوية المختلفة التي تتكون منها البكتيريا، مثل الأسواط، والكبسولة، والجدار الخلوي، والغشاء السيتوبلازمي، والسيتوبلازم. وهكذا، يلعب كل مكون دورًا في دورة حياة البكتيريا.

تتكون الخلية البكتيرية من مادة عضوية وغير عضوية تتمثل في الصيغة $C_5H_7O_2N$

تحتاج الكائنات الحية الدقيقة إلى العناصر الأساسية مثل الماء والنيتروجين والكبريت والفوسفور والمعادن والعناصر المحددة من أجل نموها. وتستند دراستها إلى أخذ العينات والمراقبة تحت المجهر

توجد الكائنات الحية الدقيقة في جميع النظم البيئية: في التربة، في البيئات المائية، في الهواء، وفي مياه الصرف الصحي

وهي تلعب دورًا بالغ الأهمية في البيئة، مثل دورة النيتروجين (N) و الكربون © و الاكسجين (O) و الكبريت (S) و الفسفور (P)

تتمتع البكتيريا بالقدرة على تحلل البروتينات إلى أحماض أمينية ، والكربوهيدرات الى سكريات و الدهون الى احماض دهنية $(CH_3\text{-}(CH_2)\ n\text{-}COOH)$ ، كما تتحلل النفايات السليلوزية والهيدروكنات بيولوجيًا بفعل سلالات معينة من البكتيريا و الفطريات. . يخضع كل مركب لعدة مراحل من التحلل البيولوجي

تُعرف عملية التحلل باسم «علم الإنزيمات»، وتتميز بحركية الإنزيمات (فرضية ميكاليليس-منتن)، التي تدرس . سرعة التفاعلات الكيميائية أو الكيميائية الحيوية، حيث يقوم الإنزيم (E) بتحويل الركيزة (S) إلى ناتج (P) .دون تغيير حالة التوازن .

الكلمات المفتاحية: *علم الأحياء الدقيقة البيئية، الكائنات الدقيق ة،بيوالكيمياء الحيوية البيئية، التحلل البيولوجي، علم* الانزمات.

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General introduction

Environmental microbiology and biochemistry are two complementary and interdependent disciplines.

The first focuses on the biodiversity of microorganisms across the different parts of the biosphere, as well as their structure, lifestyle and role in ecosystems functioning.

Whereas the second explains metabolism, which includes all the chemical transformations (synthesis and degradation reactions) that support the production of bacterial components. These reactions are catalyzed by specific enzymes. Bacterial metabolism reveals the biochemical and molecular characteristics that enable microorganisms to be classified.

Their main interactions are based on:

- Biogeochemical cycles, which involve the transformation of elements in soil, water and air through microorganisms.
- Microbiology identifies bacteria capable of degrading pollutants, while biochemistry studies the specific metabolic and enzymatic pathways involved in this degradation.

Both fields are applied in the management of various waste types, in soil fertilization through humus formation, and consequently in site restoration.

Part I of this manuscript outlines the fundamental structure of the bacterial cell and its physiology (nutrition and growth), as well as the role of microorganisms in the biogeochemical cycle, along with an overview of microbiology in soil, aquatic environments, air and wastewater.

Particular emphasis is given to the properties of microbial ecosystems and microbial biodiversity.

Part II focuses on the molecular composition of the cell, including the structure of proteins, lipids and carbohydrates, as well as the enzymatic reactions occurring among the various molecules.

This section also introduces biogeochemical cycles and highlights the role of microorganisms in the degradation of proteins, cellulosic waste and petroleum residues.

Part I :

Environmental Microbiology

I.1 Introduction

Environmental microbiology is a science that examines microbial biodiversity and abundance across different ecosystems such as water, air, and soil, as well as the importance and role of microorganisms within their natural environments.

It focuses on the interactions between microorganisms, as well as between them and their environment. Microorganisms play a vital role for ecosystem health and functioning, carrying out key processes such as waste decomposition, mineral ion solubilisation, production of organic compounds, and nitrogen fixation.

Microbiology is closely associated with microbial ecology, which examines the interactions between microorganisms and their surrounding environment.

The significance of environmental microbiology lies in the following aspects:

- The essential role of microorganisms in major biogeochemical cycles; although microscopic in size (µm) they are extremely abundant.
- Interactions between microorganisms, including competition, symbiosis, and predation.
- Interactions between microorganisms, and plants, and animals, such as those occurring in rhizosphere and within gut flora).

Microorganisms are living organisms of microscopic size, measured in micrometers. They exhibit diverse morphologies and are classified into families of living organisms:

Bacteria: these are single-celled organisms lacking a nucleus (prokaryotes).

Fungi: these are eukaryotic organisms with a nucleus, including yeasts and molds.

Protozoa: mobile, single-celled eukaryotic organisms.

Microalgae: these are photosynthetic eukaryotes.

Viruses: extremely small infectious organisms that require a host for replication.

The study of each major categories of microorganisms constitutes a specialized field. Consequently, microbiology is a multidisciplinary science comprising bacteriology, mycology, protozoology, algology and virology.

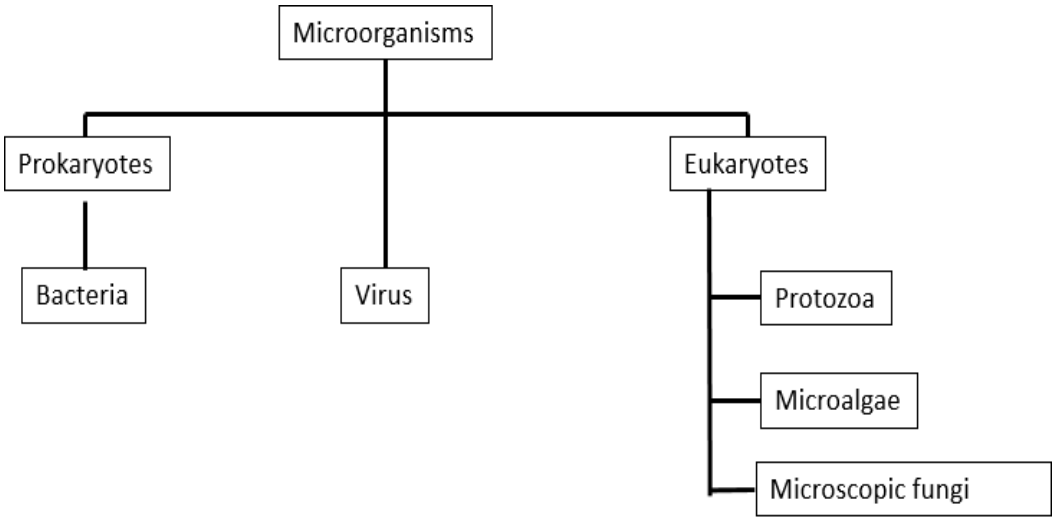


Figure I.1: The different types of microorganisms

The classification of microorganisms is based on three fundamental and interconnected principles:

- **Classification:** grouping according to common characteristics.
- **Nomenclature:** assigning a name in accordance with international rules.
- **Identification:** determining whether an unknown sample belongs to an existing group.

Today, identification methods have evolved, and taxonomy is referred as 'polyphasic' since it integrates several complementary approaches:

-**Phenotypic criteria**: morphology, nutritional requirements and metabolism.

-**Genotypic criteria**: DNA analysis.

-**Binomial nomenclature**: each microorganism is named using its genus (capitalised) and species (lowercase), both written in italics.

Example: *Staphylococcus aureus*

I.2 Morphology and functional anatomy of bacteria

I.2.1 Morphology of bacteria

Bacterial morphology is determined by the shape of the cells, their size, and the arrangements they establish with one another [1,2].

a- Size

Bacteria are situated between viruses and unicellular algae or protozoa. The smallest bacteria, such as Chlamydia measure approximately 0.2 µm in size, whereas some spirochetes may attain lengths of up to 250 µm. In general, bacterial size typically ranges from 1 to 10 µm.

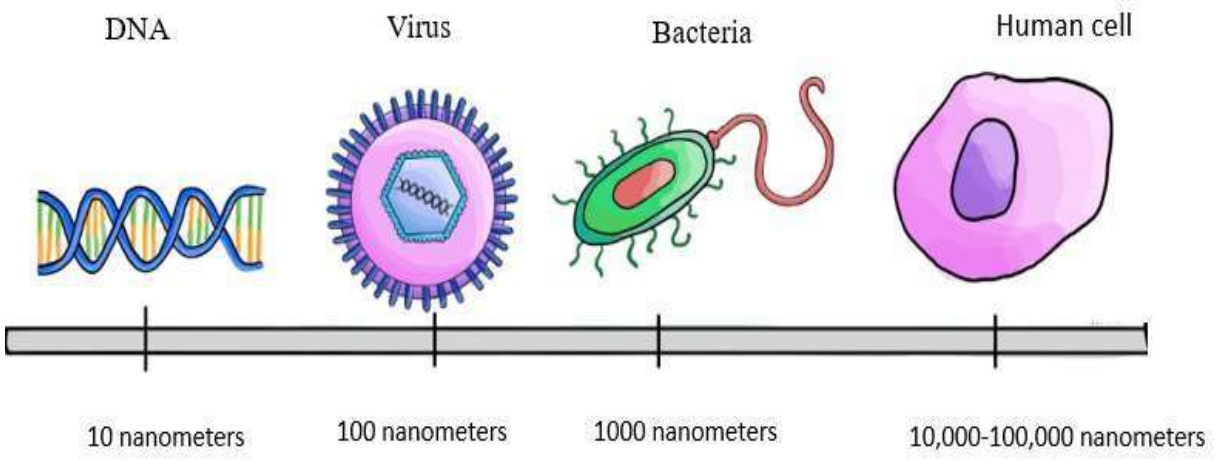


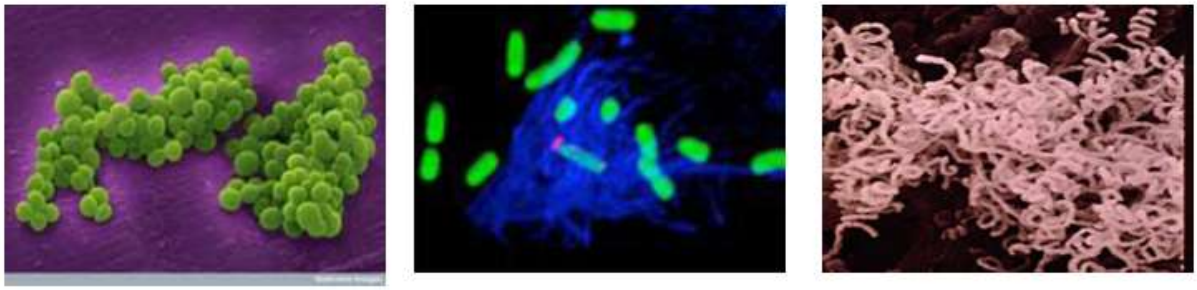
Figure I.2: The size of bacteria compared to other cells and their components

b- Shape

Bacteria exhibit a great diversity of shapes. The three main shapes are: spherical or coccal, cylindrical or rod-shaped, and spiral or helical.

- Spherical shape: round or coccus-shaped bacteria, occurring singly, in chains or in clusters (varying numbers of cells), such as Staphylococci, Streptococci (Figure 2-a).
- Rod-shaped: these are elongated bacterial cells known as 'bacilli', with rounded ends. Some bacilli taper at both ends 'fusiform bacilli' or are curved (vibrio) (Figure 2-b).
- Ovoid shape: an intermediate form between cocci and bacilli; cells of this shape are known as "coccobacilli".

- Spiral shape: Microorganisms of this type have a helical, extremely elongated body. There are rigid spiral cells known as ‘spirilla’ and flexible spiral cells known as ‘spirochetes’ (Figure 2-c).
- Other forms: These include the pedunculate form characteristic of Caulobacter, the filamentous form of iron-oxidising bacteria or Sphaerotilus, and finally the mycelial forms.



a : Spherical b : Stick c : Spiraled

Figure I.3: The different shapes of bacteria

c- Cell arrangements

A bacterial species may exist as individual isolated cells or form characteristic arrangements specific to the species, such as pairs, regular clusters, chains, or groups of four cells known as tetrads.

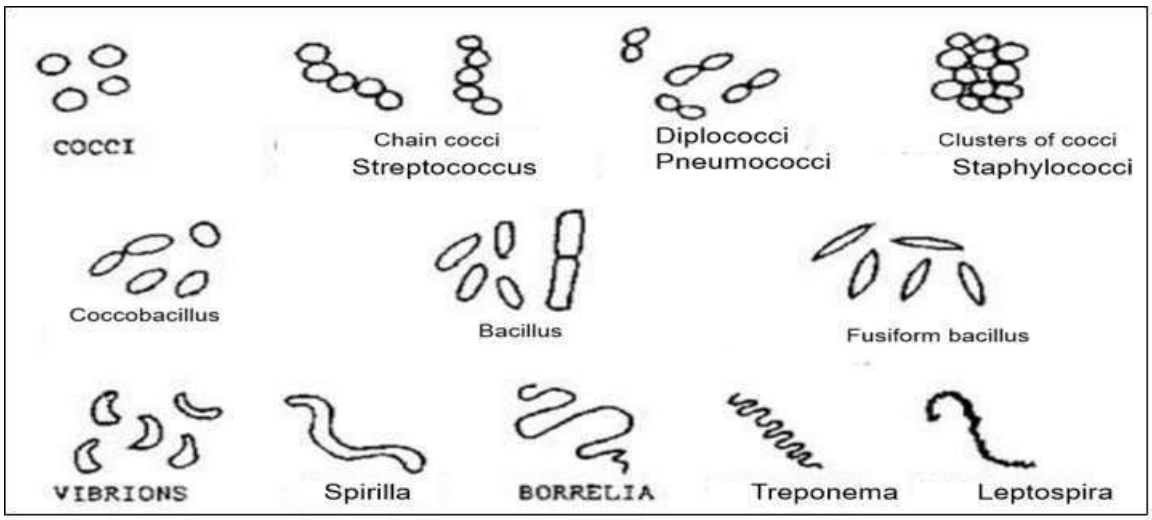


Figure I.4 : Different bacterial forms and communities

I.2.2 Functional anatomy of bacteria

The diagram below shows the anatomy of a bacterial cell. Some structures are common to all bacteria and are referred to as « constant » elements, whereas others are present only in certain bacteria and are termed « variable » or « facultative » elements. From the outside to the interior, the bacterial cell consists of:

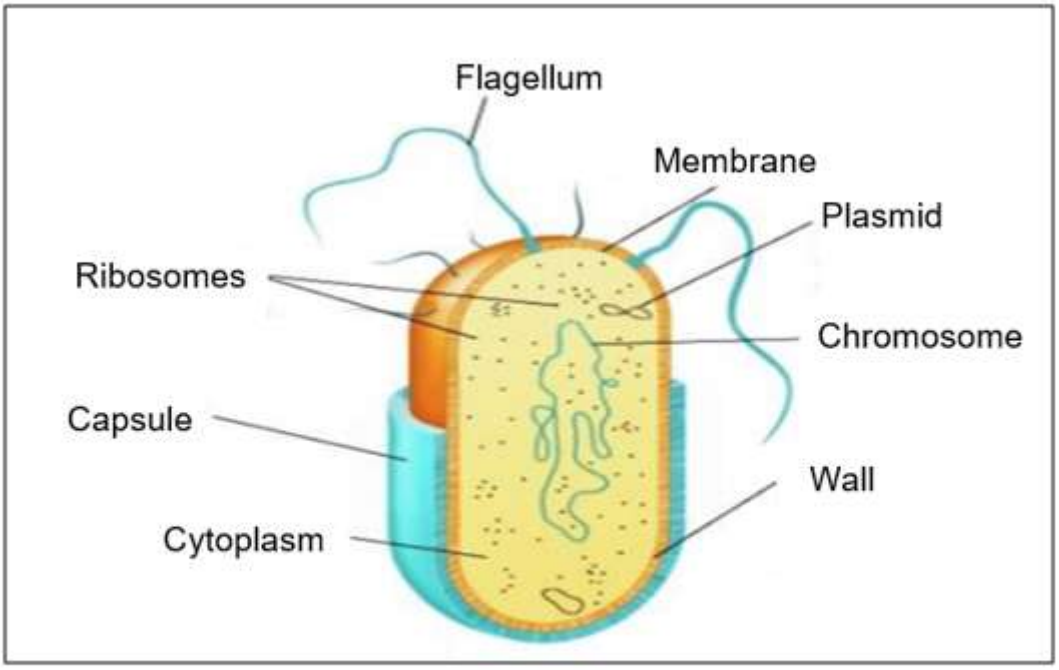


Figure I.5: Diagram of the structure of a bacterial cell [1]

I.2.2.1 Flagella

These are locomotive appendages located outside the plasma membrane and the cell wall. They are thin filamentous structures, approximately 20 nm in diameter and 15 to 20 µm in length.

-Different types of insertion

Bacterial species are often distinguished by the arrangement of their flagella. Several arrangements are possible

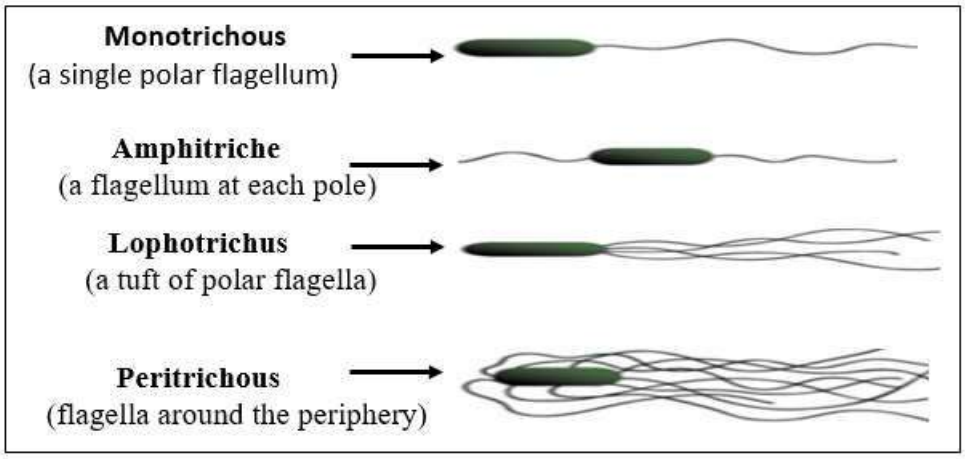


Figure I.6 : Diagram showing the insertion of flagella

-Functions of the flagellum

Locomotion: This is achieved through a rotating or beating motion.

Chemotaxis: Certain substances act as attractants for motile bacteria, such as sugars and amino acids, whilst others exert a repellent effect, such as phenol.

I.2.2.2 The capsule

Many bacteria produce viscous organic substances that form a more or less compact layer around their cell wall.

This is an additional, gelatinous-mucous layer, typically composed of polysaccharide and occasionally polypeptide. It can be observed using China ink staining.

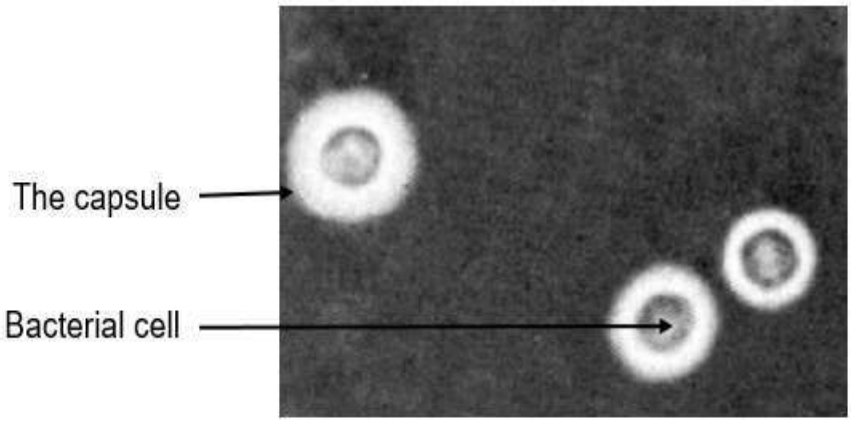


Figure I.7 : Highlighting the capsule using the "China ink" method

- Capsule function

It is not essential for the cell survival; bacteria can live without a capsule. However, the capsule provides certain advantages by performing the following roles:

Protection: against ultraviolet radiation, desiccation, as well as various physical and chemical agents,

Virulence (pathogenicity): It counteracts phagocytosis by reducing bacteria adhesion to macrophages.

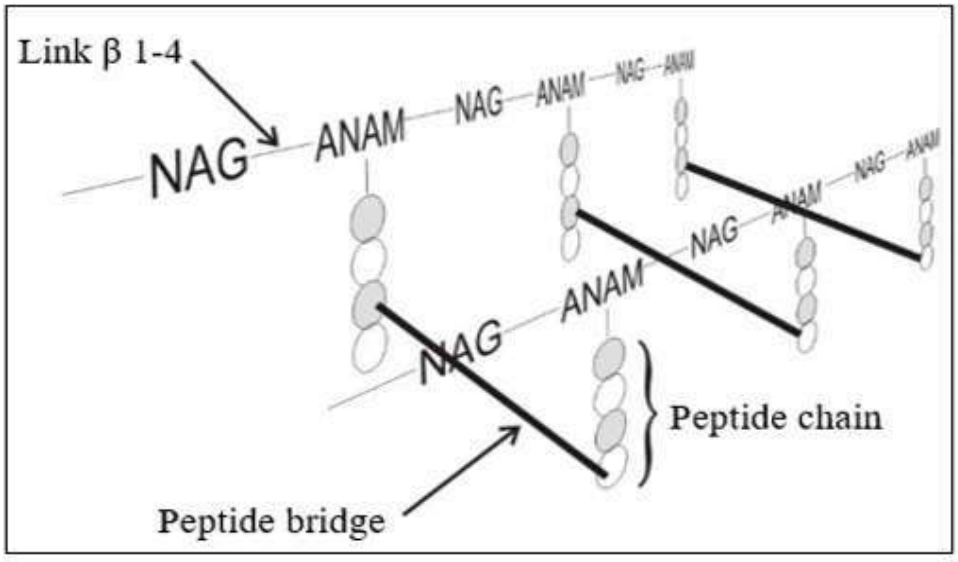
It exerts a negative chemotactic effect on leukocytes.

I.2.2.3 The cell wall

The cell wall acts as a true exoskeleton, preserving the structural integrity of the bacterial cell. It determines cell shape and enables it to withstand the high internal osmotic pressure, which ranges from 5 to 20 atmospheres.

It accounts for 20% of the dry weight of the bacterial cell.

The cell wall is composed of a heteropolymeric glycopeptide in all bacteria. However, there are structural differences between the cell walls of Gram-positive and Gram-negative bacteria.



NAG: N-acetylglucosamine

ANAM: N-acetylmuramic acid

Figure I.8 : Network structure of peptidoglycan

This differentiates bacteria into two groups allows two types of bacteria to be identified: Gram-negative and Gram-positive bacteria.

The table below presents the chemical composition of the cell wall.

Table I.1 : Comparison of the overall chemical composition of the cell wall in Gram⁺ and Gram- bacteria

Compounds	Gram+	Gram-
Osamines	Abundant	Little
Amino acids %	24-35%	≈ 50%
Number of amino acids	4 -10	16-17
Teichoic acids	Present	Absent
Dare	20-60%	20-60%
Lipids	1-2,5	10-22%

I.2.2.4 The periplasmic space

It contains enzymes such as hydrolases involved in nutrition, as well as proteins responsible for transporting molecules into the cell.

Gram-positive bacteria release enzymes outside the cell, where they are referred to as «exoenzymes ».

In Gram-negative bacteria, these enzymes are retained in the periplasmic space between the inner and outer membranes.

I.2.2.5 Cytoplasmic membrane

The plasma membrane is a flexible, semi-permeable membrane measuring approximately 40 to 80 Å in thickness that surrounds the cytoplasm. It consists of two distinct layers: an external hydrophilic layer and an internal hydrophobic layer.

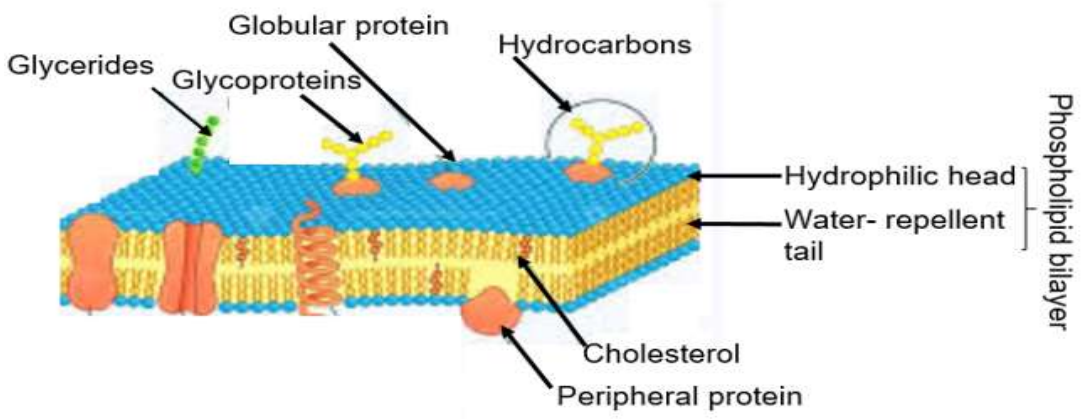


Figure I.9: Structure of the plasma membrane

-Chemical Composition

The bilayer consists of phospholipids (30–40%) with incorporated proteins (60–70%). Cations such as Ca^{2+} and Mg^{2+} contribute to the stabilization of the plasma membrane.

The plasma membrane contains the enzymes of the respiratory chain; as well as other enzymes involved in lipid synthesis and DNA replication.

-Function

It regulates the selective exchange of chemical substances and contains respiratory enzymes that catalyze redox reactions. It is at this level that the bacterium produces its energy.

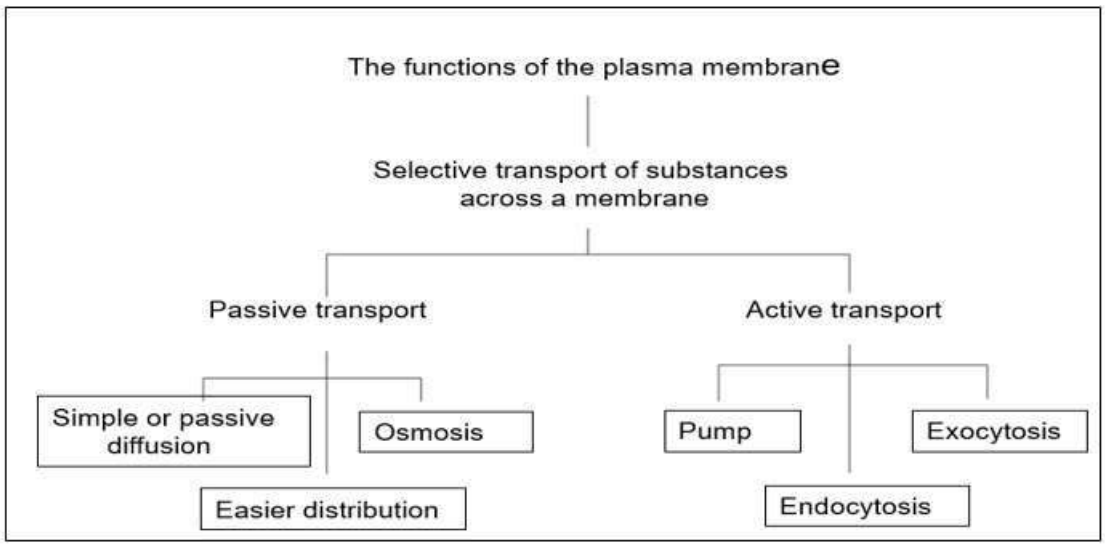


Figure I.10 : The various functions of the plasma membrane

I.2.2.6 The cytoplasm

It is a colloidal hydrogel with a pH ranging from 7 to 7.2, composed of the various constituents of the bacterial cell, including genetic material, storage substances, specific specialised organelles, and ribosomes responsible for protein synthesis.

I.3 Bacterial physiology

This science studies of the functions of bacteria such as nutrition, respiration, reproduction, etc.) and other processes related to their normal functioning.

I.3.1 Bacterial nutrition

To satisfy their nutritional needs, microorganisms must obtain two categories of substances from their environment: energy-yielding substances, required for the synthesis of cellular components and essential elements that serve as the fundamental building blocks of the cell.

I.3.1.1 Energy source

Energy substrates or an energy source (light) as energy sources that enable the production of ATP, which is necessary for cellular biosynthesis.

According to the type of energy utilised, microorganisms, classified into two categories:

- Phototrophs or photosynthetic organisms, obtain their energy from light.
- Chemotrophs or chemosynthetic organisms, obtain their energy from the oxidation of organic or inorganic chemicals.

I.3.1.2 Basic components:

These are the substances that constitute the cell.

- Water

It represents 80-90 % of the mass of a bacterial cell and is found in all environments. Water also provides bacteria with a source of hydrogen and oxygen.

-Carbon source

An adequate supply of carbon is necessary for bacteria, as it is an essential constituent of the cell. Bacteria are classified into two categories:

- **Autotrophs**: These organisms can develop in inorganic environments where carbon dioxide (CO_2) serves as the sole source of carbon.

- **Heterotrophs**: These organisms depend on e organic compounds as a carbon source and are able to degrade various hydrocarbon substances such as alcohol, acetic acid and lactic acid.

-Source of nitrogen

Microorganisms require nitrogenous compounds for protein synthesis, as proteins represent approximately 10% of their dry mass. Certain bacteria are able to fix nitrogen in its simplest form, namely molecular nitrogen ».

Other inorganic compounds such as nitrites and nitrates may also be used,

Nitrogen can likewise be obtained from organic sources through the amine groups of organic compounds (R-NH_2), either in the form of ammonia, released after deamination or through the NH_2 group via transamination.

-Source of sulphur and phosphorus

Sulphur and phosphorus are essential mineral components of bacterial cells.

Sulphur is found in certain amino acids and is incorporated either as sulphate or from organic sulphur-containing compounds.

Phosphorus, on the other hand, forms part of nucleic acids, numerous coenzymes and ATP, and is taken up by cell in the form of inorganic phosphate.

-Mineral elements

This category of elements includes Na, Mg and Cl. They are constituents of various enzymes and coenzymes, such as Fe in cytochrome, and therefore contribute significantly to maintaining physico-chemical equilibrium.

-Trace elements

These elements occur in very small amounts as trace elements, including magnesium, calcium, cobalt, copper and manganese. They act as cofactors or enzyme activators.

I.3.1.3 Specific requirements

In addition to these basic elements, some bacteria require, organic compounds for growth, that they cannot synthesise themselves; these are referred to as growth factors.

These growth factors are divided into three categories of substances:

- **Amino acids**: these function as growth factors because they are essential for protein synthesis.
- **Purine and pyrimidine bases**: these are components of nucleic acids and are therefore essential for their formation.
- **Vitamins**: these act either coenzymes or as precursors of coenzymes. In their absence, the bacterium is unable to perform the corresponding function.

Their first of their common characteristic is their effectiveness at very low concentrations. Approximate requirements are around 25 mg /L, for amino acids, 10 mg /L for purine bases, and 1 to 24 µg /L for vitamins.

Their second characteristic is their strong specificity.

Example: Nicotinamide is one of the components of the NAD molecule. Even a slight modification in the position of the $CO\text{-}NH_2$ group on the benzene ring, or its substitution with a neighboring group such as COOH or CH, makes the compound entirely inactive.

In this context, a distinction is made between:

- **Prototrophic bacteria**: These are bacteria that do not require growth factors for their development.
- **Auxotrophic bacteria**: These require growth factors for their development.

I.3.2 Bacterial growth

Growth is defined as the orderly increase in all the components of an organism.

In unicellular microorganisms, it leads to an increase in cells size, mass, volume and the number. It may also be considered as a series of anabolic and catabolic reactions.

In liquid culture, growth is monitored by measuring the increase in living mass (biomass). It is characterised by a series of growth phases and defined by parameters such as the maximum specific growth rate (or growth rate) and the generation time.

I.3.2.1 Bacterial replication

The completion of bacterial chromosome replication is followed by cell division, a process during which the cell elongates and new membrane and cell wall material is synthesised. The resulting daughter cells are arranged in a specific pattern and retain the same morphology, structural features, and physiological and physicochemical properties as the parent cell. Thus, a single cell gives rise to two daughter cells, each of which will subsequently divide to produce two additional cells in the next cycle. This mode of multiplication follows a geometric progression.

$$1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow 32 \text{ ect}$$

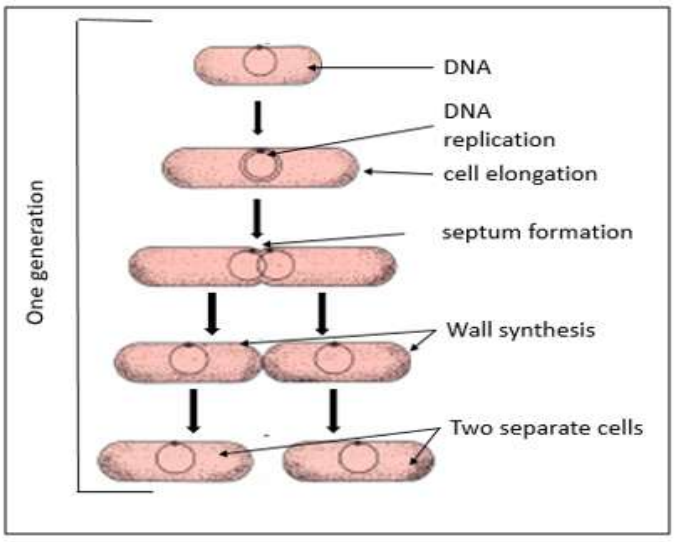


Figure I.11: The mechanism of bacterial reproduction

-Generation time (G)

Generation time is the period required for a bacterium to divide. It can be expressed as the time required for the cell population to double in number or equivalently, for the biomass to increase twofold.

$$G = \frac{t}{n} \qquad (Eq.1)$$

G: is the generation

n: is the number of divisions

t: is the known time

Each bacterial species is characterised by its generation time (G) under optimal and ideal growth conditions.

Example:

E. coli: 20 minutes

L. acidophilus: 100 minutes

-Growth rate

The hourly growth rate (R) of bacteria is defined by the formula below:

$$R = \frac{n}{t} = \frac{1}{G} \qquad (\text{Eq. } 2)$$

The Neperian growth rate (μ) is given by the following formula:

$$\mu = R.\ln 2 = \frac{1}{G}\ln 2 \qquad (\text{Eq. } 3)$$

I.3.2.2 Growth curve

Bacterial growth is represented graphically by ln(N)=f(t) plot, known as the « growth curve », which consists of several phases.

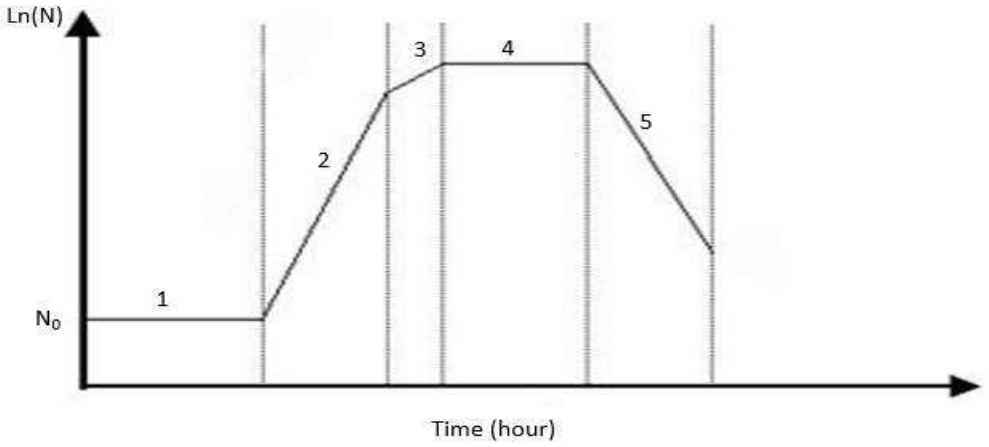


Figure I.12 : Bacterial growth curve

- **Latent phase 1**: During this stage, bacteria adapt to their new environment and synthesise the enzymes required for substrate utilisation. This phase is characterised by: μ =0 and N =No

- **Exponential phase 2**: The cell reproduction rate reaches its maximum during this phase. (μ) expo is at its maximum and remains constant, resulting in a continuous increase in the bacterial population (N).

- **Steady-state phase 3**: This phase is characterized by the exhaustion of available nutrients, the buildup of toxic metabolic by-products, and adverse changes in environmental conditions, particularly pH. As a result, bacterial growth ceases, and the cell population (N) remains stable.

- **Decline phase 4**: During this stage, bacterial cell division ceases due to the complete exhaustion. Cell death progressively exceeds cell reproduction, resulting in a negative specific growth rate (μ) and a decrease in the bacterial population (N).

-Bacterial cell growth yield

The effectiveness of nutrients conversion into cellular biomass by the yield coefficient, which reflects the amount of biomass produced from a given quantity of nutrients consumed.:

$$Y=\frac{mass\ of\ bacteria\ produced\ (g)}{mass\ of\ substracte\ consumed\ (g\ or\ mol)}$$

I.4 The role of microorganisms in the cycle of bioelements

The cycle of living matter refers to the continuous cyclical chemical transformations undergone by the elements that constitute living organisms, such as carbon, nitrogen, sulfur and phosphorus compounds. These cyclic processes occur within the biosphere, which comprises terrestrial surfaces (soils), the lower layers of the atmosphere, and aquatic environments such as oceans [4].

- ✓ **Carbon and microorganisms**

Carbon exists in nature primarily as carbon dioxide (CO_2), carbonates and hydrogen carbonates. Microorganisms contribute significantly to the decomposition carbon-rich organic matter derived of dead plants and animal., Numerous specialized aerobic and anaerobic microorganisms, including species of Pseudomonas, cellulolytic, pectinolytic bacteria, denitrifying bacteria, actinomycetes, and Bacillus. participate in the decomposition of organic materials, particularly those of plant origin. This biodegradation process leads to the formation of humus.

In aquatic sediments, as well as at the bottoms of rivers and ponds, organic matter may undergo anaerobic mineralization through fermentation processes.

- ✓ **Nitrogen and microorganisms**

The microbial degradation of nitrogen-containing compounds proceeds through a series of stages involving different groups of microorganisms. During aerobic autotrophic nitrification, Nitrosomonas species convert ammonia into nitrite, while Nitrobacter species subsequently oxidize nitrite into nitrate.

$$NH_4^+ + 3/2\,O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + H_2O + 2H^+ \text{ (Nitritation)}$$

$$NO_2^- + 1/2\,O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad \text{(Nitratation)}$$

The nitrates produced during nitrification can serve as terminal electron acceptors in absence of oxygen. The bacteria involved in denitrification belong to genera such as Pseudomonas, Acinetobacter, etc., and are heterotrophic.

$$NO_3^- + 2H_3O^+ + 2é \longrightarrow NO_2^- + 3H_2O$$

$$NO_2^- + 4H_3O^+ + 3é \longrightarrow 1/2N_2 + 6H_2O$$

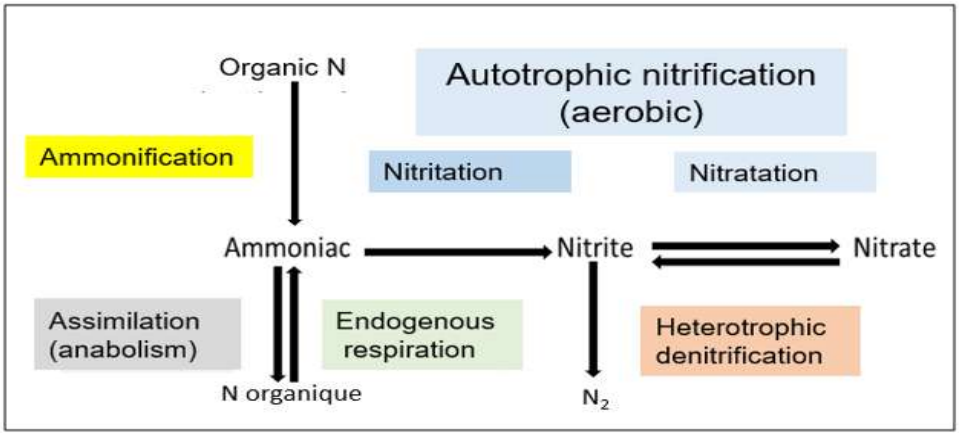


Figure I.13 : The nitrogen degradation process

- ✓ **Sulphur and microorganisms**

Following the death of plants and animals, microorganisms decompose organic sulfur-containing compounds, resulting in the production of hydrogen sulfide (H_2S), a gas characterized by its unpleasant odor.

Within the biosphere, H_2S is subsequently oxidized to sulfate by aerobic microorganisms such as *Thiobacillus thiooxidans*, as well as by anaerobic microorganisms

including purple and green sulfur bacteria. The resulting sulfate is then assimilated by plants, thereby completing the sulfur cycle.

I.5 Characteristics of microbial ecosystems

An ecosystem consists of a community of living organisms and the surrounding atmospheric, geological and soil environment. All these components interact through a complex network of interdependencies that supports and maintains life [5, 6].

They are distinguished by several key characteristics:

-Ecosystems are complex and comprise a wide diversity of species.

-They are characterised by their interactions with their surrounding environment.

-Their structure and functioning are closely interconnected.

-The ecosystem maintains overall stability

-Microorganisms reproduce rapidly and perform important roles:

- ✓ They contribute to maintaining the balance and cycling of matter within the lithosphere, atmosphere and hydrosphere.
- ✓ In terrestrial microbial ecosystems, microorganisms contribute to soil fertility.
- ✓ Microorganisms present in aquatic ecosystems contribute to water purification by decomposing organic matter a process known as self-purification.

I.5.1 Interspecific interactions

There are two main types of relationship between microorganisms and their hosts:

- ✓ **Symbiosis**

  This mode of life involves a close association between microorganisms and their host During their coexistence, where both partners coexist and gain significant benefits from the relationship.

  Example:

  The association of nitrogen-fixing bacteria with the roots of certain leguminous plants.

- ✓ **Parasitism**

This is a relationship between two parties in which one gains an advantage whilst the other derives no benefit and may be harmed. Such interactions are typical of microorganisms that are pathogenic to humans or animals, commonly referred to as pathogenic parasites.

- ✓ **Predation**

  Several predator-prey interactions exist within microbial communities. Bacteria at as a food source (prey) for protozoa and metazoans (predators), although bacteria often compete more effectively for dissolved nutrients than protozoa. Similarly, protozoa serve as food (prey) for metazoans.

I.5.2 Soil microbiology

Soil microflora refers to the microbial population found in the soil. It includes six major groups of microorganisms: bacteria, actinomycetes, fungi, algae, protozoa and viruses [7].

They play a vital and irreplaceable role within the lithosphere. The microbial population is very abundant near the surface, in aerobic conditions, and becomes scarcer as one moves away from the surface. At a depth of 2 meters, there are only 20,000 bacteria per gram of soil. Although bacteria are the most abundant organisms, their overall mass per unit area is generally lower than that of some other organisms.

Table I.2 : Example of organism populations in fertile agricultural soil [4]

Organism	Number per gram of soil
Bacteria	2 500 000 000
Aktinomycetes	700 000
Fungus	400 000
Algae	50 000
Protozoa	30 000

There are three main types of soil flora:

-**Decomposing flora**: This flora thrives near inert organic matter (animal carcasses, plant debris) buried in fresh soil and breaks it down through a series of complex chemical reactions to produce humus. The latter is valuable for the soil because it binds mineral ions from fertilizers or from the mineralizing activity of bacteria and promotes water retention.

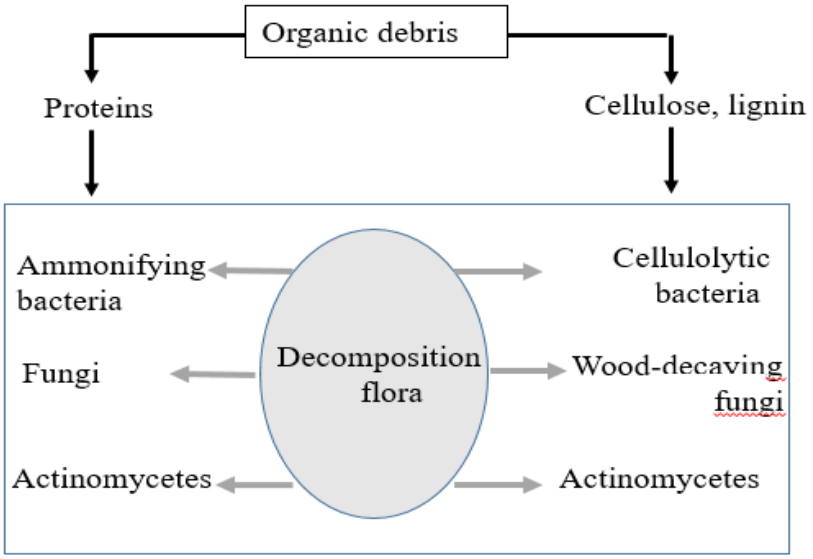


Figure I.14: Role of Decomposer Flora

-**Mineralization bacteria**: These are found in the rhizosphere (on the surface of the roots of growing or germinating plants). These plants need nitrate ions (NO_3^-) to grow.

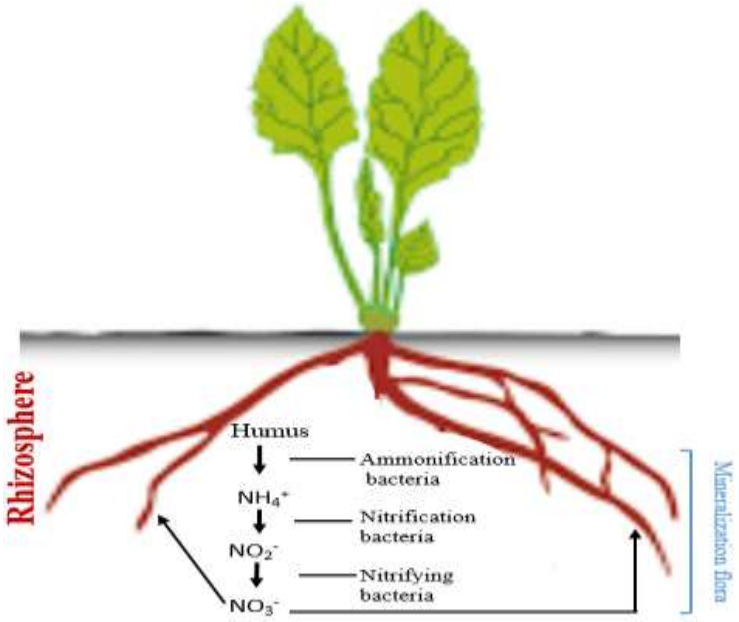


Figure I.15: Formation of the mineralizing flora

-Atmospheric nitrogen-fixing bacteria: Some bacteria have the ability to convert atmospheric nitrogen into ammonium ions or nitrates.

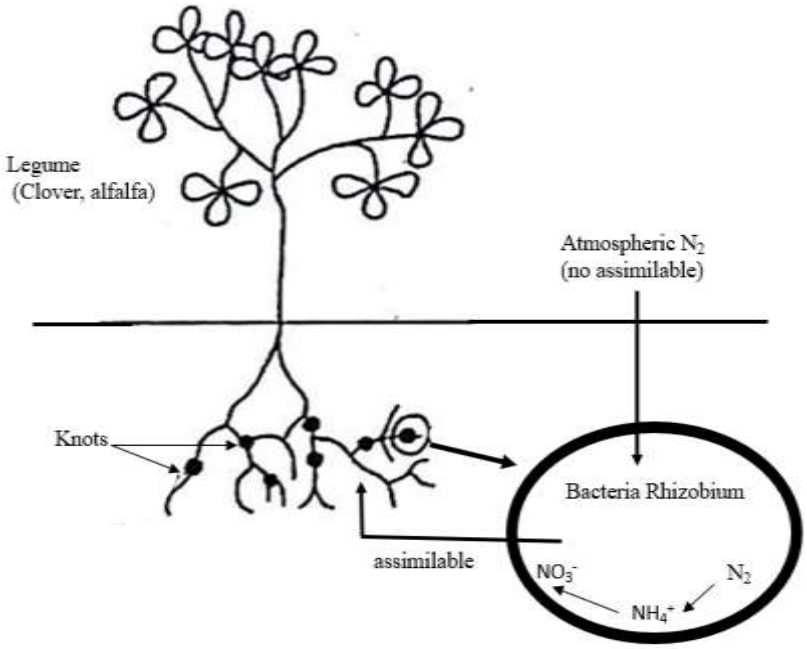


Figure 1.16: Atmospheric nitrogen fixation

•Bacteria

Bacteria are the most abundant, but their total weight per unit area is less than that of other organisms.

In general, soil bacteria are heterotrophic microorganisms that obtain their energy by decomposing organic matter. They constitute the most abundant group of microorganisms in soil. The density of the total bacterial microflora, expressed as the number of bacterial cells per gram of soil, ranges from 10^6 to 10^9.

In mineral soil, the microbial population decreases rapidly with depth on the other hand,

In organic soil, the microbial population hardly decreases with depth, and is sometimes greater at 160 cm than at the soil surface [8].

-Influence of soil factors

The behaviour, activity, and biochemical processes of soil bacterial communities are influenced by several soil factors, particularly aeration, moisture content, and temperature.

✓ **Aeration**

In well-aerated soils, bacteria and fungi predominate. In contrast, in soils with limited oxygen availability, bacteria are primarily responsible for most of the chemical and biological transformations occurring within the soil.

✓ **Moisture**

Water is a crucial environmental factor for bacteria bacterial growth and activity, as it constitutes a major component of cellular protoplasm. However, excessive moisture can impair gas exchange and reduces the amount of oxygen availability, thereby affecting bacterial metabolism.

✓ **Temperature**

Each bacterial species has an ideal growth temperature and a specific temperature range beyond which its metabolic activity and growth are inhibited.

Drying can eliminate at least 80-90% of soil microorganisms, whereas freezing results in the destruction of about 50% of them.

• Actinomycetes

Actinomycetes are filamentous, unicellular microorganisms. that rank second only to bacteria in abundance. They constitute a major component of the normal microbial population in soil as well as in lake and river sediments. Most actinomycetes are aerobic organisms.

-Influence of soil factors

The growth and activity of actinomycetes are primarily affected by the nature of organic matter, soil pH, moisture content and temperature.

Most soil actinomycetes are aerobic.

The presence of carbon influences the growth of actinomycetes; which are therefore particularly abundant in soils rich in organic matter.

These microorganisms can use carbon from both simple and complex compounds, including organic acids and sugars. NH_4^+, NO_3^- and amino acids are used as a source of nitrogen.

The pH range, most favourable to the proliferation of actinomycetes in soil is 6.5 to 8.0. They prefer moderately moist soil and exhibit optimal growth at temperatures between 28 and 37°C. Their activity is greatly reduced outside this range and is almost absent at temperatures below 5°C or above 39°C.

• **Fungi**

Fungi include two main groups: yeasts and moulds.

Yeasts are unicellular fungi measuring between 4 and 6 µm. They grow rapidly in environments rich in sugar or starchs at a pH of 4.0. Moulds, on the other hand, develop from spores that germinate and produce elongated filaments, called hyphae, the network of interconnected hyphae forms the mycelium.

The fungal population generally ranges from 10^4 to 10^6 individuals per gram of soil.

The influence of soil factors

Fungi are heterotrophs; that depend on degradable organic compounds as sources of carbon and energy. Unlike autotrophic organisms they cannot obtain the energy necessary for growth from light or from the oxidation of inorganic substances

High soil moisture plays an important role in fungal growth. Excessive moisture prevents the diffusion of molecular oxygen, which is necessary for their aerobic metabolism.

Several species of fungi flourish in highly acidic environments with pH values between 2.0 to 3.0, while other are adapted to alkaline environments where the pH can reach 9.0.

The use of ammonium-based fertilisers often stimulates fungal proliferation, as the microbial oxidation of ammonium produces nitric acid, resulting in soil acidification.

Most fungi are mesophilic. However, there are thermophilic species and grow best at temperatures of up to 50°C.

• **Algae**

Algae are abundant in soils that receive adequate moisture and light. Their populations generally range from 100 to 10,000 cells per gram of soil.

Influence of soil factors

Algae are predominantly concentrated within the upper 5–10 cm of soil, where sunlight is readily available. Algal population decreases rapidly with increasing soil depth.

Table I.3 : Effect of depth on the presence of certain bacterial species

Depth (cm)	Chlorella (species)	Chlamydomonas muscicola	Pleurococcus vulgaris
0-2,5	11 000	650	140
5	5 330	650	650
10	4 200	2 070	410
15	1 030	410	80

The growth of algae is primarily influenced by pH and moisture conditions. Each algae species exhibits optimal growth within a specific pH range.

I.5.3 Microbiology of Aquatic Environments

Bacteria are key components of the biological cycle in aquatic environments. They facilitate the biodegradation of organic matter in solution, leading to an increase in their numbers in the environment. Surface waters also support the growth of algae, which multiply through photosynthesis and the mineral salts dissolved in the water [9].

These algae subsequently become food for protozoa. Thus, the mineralization process begins with the involvement of all these microorganisms. This is the phenomenon

of self-purification, which is closely linked to the metabolism of the microorganisms present and their abundance. The level of dissolved oxygen in the water depends on the biochemical oxygen demand and the reabsorption of oxygen at the water's surface [10]

Only a small proportion of water-purifying bacteria are free-floating. They attach themselves to aquatic plants and animals, and can be found on riverbanks and the riverbed, as well as in deep silty layers.

I.5.4 Air microbiology

I.5.4.1 Presence of microorganisms

Besides soil particles and other matter, atmospheric dust naturally contains microorganisms such as viruses, bacteria, algae and protozoa [7].

In indoor environments, including homes, shops, restaurants, the abundance of microbial colonies is influenced by several factors, including, occupancy levels, ventilation, hygiene standards and air circulation.

Microorganisms have been detected at different atmospheric altitudes (Table 8).

Table N° I.4 : A few species of bacteria and fungus isolated from the air

Altitude in metres	Bacteria	Fungus
457-1372	*-Achromobacter rathonis* *-Bacillus cereus*	*Aspergillus fumigatus* *-Aspergillus niger*
1372-2286	*-Bacillus ruminatus* *-Bacillus aerosporus*	*-Aspergillus glaucus* *-Aspergillus niger*
2286-3200	*-Bacillus megatherium* *-Bacillus prausnitzii*	*-Aspergillus niger* *-Hormodendrum herbarum*
3200-4115	*-Bacillus cereus* *-Kurthia zopfii*	*-Aspergillus calyptratus* *-Hormodendrum herbarum*
4115-5030	*-Bacillus subtilis* *-Micrococcus candidus*	*-Penicillium glabrum* *-Penicillium lanosum*

Turbulent fluctuations in wind velocity play a significant role in the airborne dispersal of microorganisms.

I.5.4.2 Transmission of pathogenic organisms

Airborne microorganisms suspended in the air can survive for a few seconds, a few hours or even several months depending on environmental conditions.

Several diseases can be transmitted through the air, particularly respiratory tract infections. The causative agents of these infections include bacteria (8.2%), viruses (19.9%) and unknown aetiological agents (71.9%).

I.5.4.3 Techniques for the removal of microorganisms

Several techniques can be employed to eliminate or reduce the concentration of microorganisms in the air, including [8]:

- ✓ **Ultraviolet radiation**

The range of the electromagnetic spectrum between 2500 and 2600 Å is particularly effective for its bactericidal properties.

- ✓ **Aerosols**

Aerosols contain bactericidal chemicals capable of reducing airborne microbial populations. These products must be free from any substances that may be toxic or irritating to humans.

- ✓ **Filters**

The effectiveness of filtration depends on several factors, including the airflow rate and properties of the filter; the type, and the size of the microorganisms being retained [10]

Table N° I.5 : Performance range of various devices for the removal of biological particles (1-5µ) from the air

Removal device	Expected percentage of bacteria removal
- **Ultra-high output filters**	99,99 +
- **High-efficiency filters**	90-99
- **Average yield filters**	60-90
- **Coarse filters**	10-60
- **Air washers and purifiers**	20-90
- **Electrostatic precipitators**	60-90

I.5.5 Microbiology of domestic water and wastewater

The microbial community present in urban wastewater consists of both saprophytic or pathogenic microorganisms originating from the the faeces and urine of humans and animals [11, 12, 13].

The density of these microorganisms in wastewater fluctuates daily and seasonally. It is influenced by factors such as size and characteristics of the population served by the sewer network, the volume of water used for waste transport, and the health condition of the individuals generating the wastewater.

Table N° I.6 : Microorganisms in human faeces and the faeces of certain animals
H : human ; **D** : dog ; **C** : cow

Micoorganisms	Excretor	Average quantity per gram of faeces
Escherichia coli	H D C	10^7- 10^9 10^6- 10^7 10^5-10^6
Bactéroides fragilis	H D C	10^8- 10^{10} 10^8- 10^9
Faecal streptococci	H D C	10^5-10^6 10^8- 10^9 10^5- 10^6
Clostridium perfringens	H D C	10^6- 10^7 10^8- 10^9 10^2- 10^3

Waterborne microorganisms frequently associated with suspended particles, either through aggregation or adsorption, resulting in considerable variations in their concentrations [14].

I.5.5.1 The state of microorganisms in wastewater

As faecal matter is carried along by the water, mechanical shear forces fragment it into particles of different sizes. However, these forces are insufficient to fully homogenise the and produce a uniform bacterial suspension. Consequently, wastewater contains particles weighing a few tenths of a milligram, each containing approximately 10 to 10^3 bacteria [15].

The, electrically charged bacteria that remain free in the water tend to adsorb onto suspended particles particularly those of a clay-like or other similar nature.

A similar phenomenon occurs with viruses. As between 3% and 100% of viral particles can become adsorbed onto suspended solids. Viruses are constantly present in wastewater and their concentrations varies in the same way to that of bacteria indicative of faecal contamination.

The adsorption and/or aggregation of microorganisms has significant consequences:

• Extend their survival time either by protecting them from inhibitory environmental factors or by enabling them to find biodegradable organic matter on the substrate.

•Oxidation does not occur properly during wastewater treatment.

•Sedimentation of microorganisms along with their carrier material.

I.5.5.2 Variations in microorganism fluxes

Microorganism concentrations in urban wastewater vary over time and space, exhibiting variations at hourly, daily, weekly, seasonal, and geographical scales [16].

Each site has its own characteristics depending on:

- Type of sewerage system (combined or separate).

The discharge of wastewater from sources containing high concentrations of toxic substances or heavily contaminated with pathogenic bacteria, effluents originating from healthcare facilities and hospitals.

•The standard of living of the population, which strongly influences water consumption patterns and is closely associated with socio-cultural factors. Moreover, significant correlations have been observed between bacterial concentrations and several physicochemical parameters such as suspended solids, chemical oxygen demand and biochemical oxygen demand [17].

I.6 Study of microbial biodiversity

Microorganisms constitute the largest component of the Earth's biodiversity. Despite their abundance, their total numbers remain difficult to estimate, and their population dynamics are challenging to characterise. Diverse microbial populations, each performing specific and complementary physiological functions and processes, interact within complex communities that are integrated into their environment.

An ecosystem is composed of a community of living organisms and its surrounding atmospheric, geological and soil environment. Within this system, are interconnected through a complex network of interactions and dependencies that support and maintain life. The biodiversity of an ecosystem enables us to study the roles of microorganisms and the nature of their interactions, including both intraspecific and interspecific relationships [15].

Microbial biodiversity includes bacteria, algae, fungi and viruses found inhabiting a wide range of environments. Collectively, these organisms play an important role in soil biological processes and in in the regulation of major biogeochemical cycles (C, O, P, N, S, etc.).

I.6.1 Sampling

Sampling refers to the collection of a representative portion of a material or substance for subsequent laboratory analysis. The use of validated or standardized sampling procedures is essential to ensure the reliability and accuracy of analytical results. Proper sampling practices are critical for obtaining representative samples that accurately reflect the characteristics of the original material, thereby ensuring meaningful and dependable laboratory analyses.

Sampling procedures vary according to the objectives of the study and the characteristics of the material being collected. The nature of the sample, whether it is water,

soil, food, or another type of matrix, plays a key role in determining the appropriate sampling method. This stage is of critical importance as it represents the initial step in a series of steps required to perform a microbiological analysis. Microbiological sampling is conducted to provide an accurate assessment of the microbiological quality of a sample. It serves to determine whether a product, process, meets established quality standards and sanitary requirements, thereby ensuring its safety and compliance with regulatory specifications.

Precautions to be taken during sampling

To avoid any risk of sample contamination during handling, several essential precautions must be strictly observed throughout the sampling process. Among them:

- Sterile sampling containers should be supplied by the testing laboratory to prevent contamination.
- The sample volume must be adequate to perform all the required analyses and, if needed, allow for repeat testing to verify the results.
- Avoid filling the containers completely and leave a 2 cm space between the surface of the substance and the lid. This facilitate proper homogenization prior to analysis.
- Containers must be sealed tightly after Sampling-Containers must be labelled at the time of sampling
- Regardless of the method used, it is important to use sterilised equipment and aseptic technique to minimise the risk of accidental sample contamination.
- Ensure that transport conditions and time limits are adhered to.
- Samples must be transported in insulated coolers and maintained at 4°C from the time of collection until their arrival at the laboratory. Upon receipt, the samples should either be analysed immediately or stored in the refrigerator until analysis is performed.

I.6.2 Microscopy

I.6.2.1 General principles

Microscopy is a set of techniques for imaging small objects. It enables the observation and imaging of biological structures and the visualisation of features that are invisible to the naked eye, either because of their colour or their size. The instrument used for observation is the microscope. The units of measurement used in microscopy are µ, µm, nm and Å.

The eye is capable of distinguishing particles with a diameter of up to 0.1 µm. However, these particles must be at least 1 µm apart.

It works by sending a wave onto the specimen. This wave is captured by the objective lens, which focuses it, and then passes through an eyepiece, creating a visible image. This image can be viewed with the naked eye, photographed, or recorded by a camera and stored on a computer for later study or analysis.

I.6.2.2 Types of microscopy

Microscopy is classified into two main types: optical microscopy and electron microscopy.

a- Optical (photonics) microscopy

This type of microscopy magnifies the optical image of a small object using optical lenses, whilst controlling the light beam to illuminate the sample. The best optical microscopes are limited to a magnification of 2,000 times.

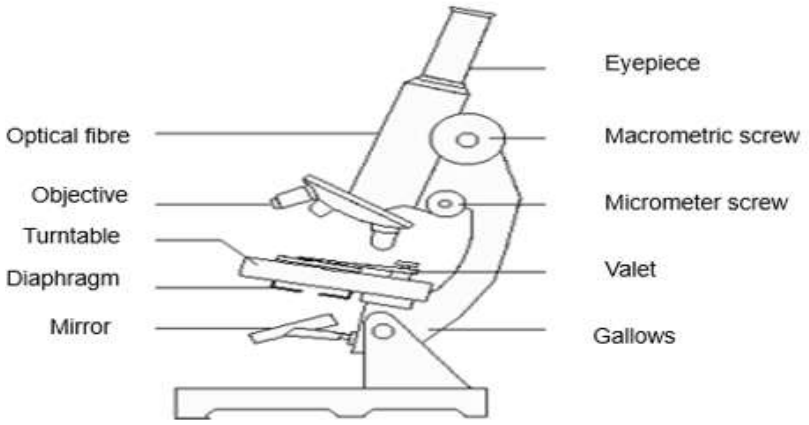


Figure I.17 : Optical microscope

There are several types of photonic microscopes:

- **The bright-field/dark-field microscope**

It is used to enhance the contrast of transparent specimens and to observe living, unstained, moving structures such as single-celled organisms. The specimen appears bright, whilst the rest of the field of view appears black.

- **Phase-contrast microscopy**

A phase-contrast microscope is an instrument that uses a condenser with an annular stop and a special objective lens with a phase plate to shift the phase of the light and create interference. It converts the minute differences in the sample's refractive index into detectable variations in light intensity (contrast).

It allows transparent, unstained samples to be observed in their natural environment. The resulting image is high-contrast and often has a slight halo around the structures.

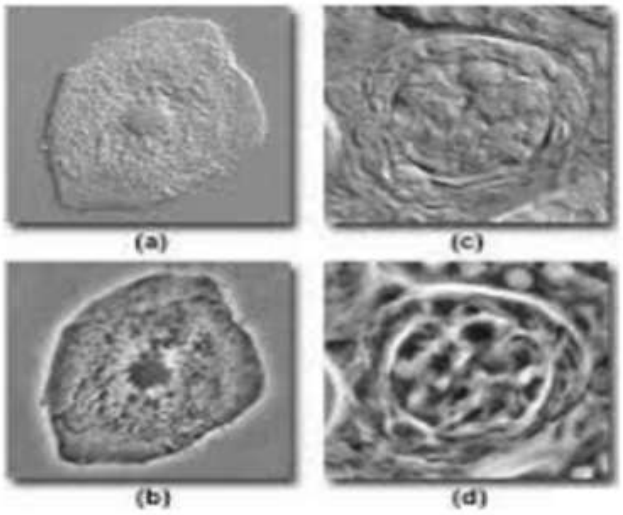


Figure I.18: of cells observed under a phase-contrast microscope

- **Fluorescence microscopy**

The sample is exposed to ultraviolet, violet or blue light which excites fluorescent dyes known as a fluorochrome, causing them to emit visible light. The most commonly used dyes are rhodamine and fluorescein Example. Rhodamine emits red light whereas fluorescein green light.

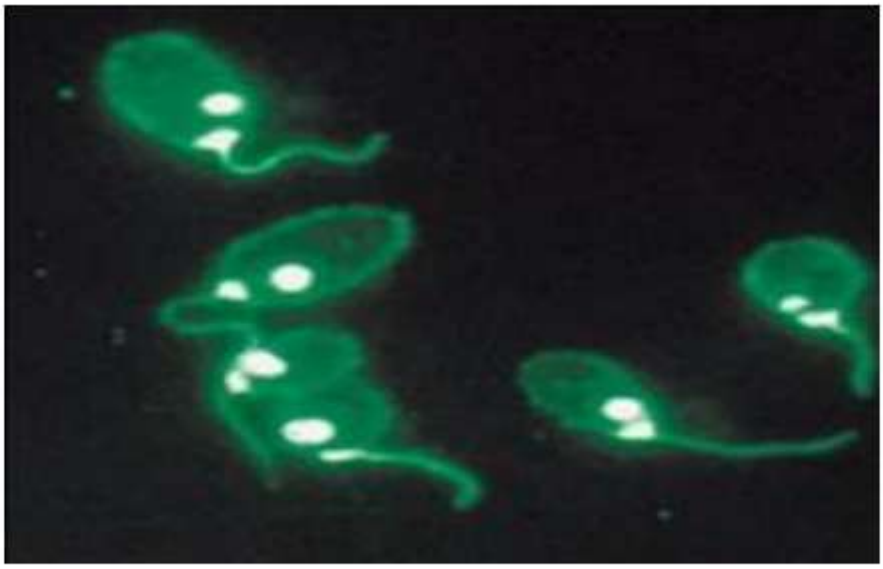


Figure I.19: Flagellated protozoan observed under a fluorescence microscope

b- Electron microscopy

The electron microscope employs electrostatic and magnetic lenses to focus and form images using a beam of electrons instead of visible light. This technique provides extremely high resolution, enabling the visualization of the ultrastructure of the samples and allowing much more detailed observation.

Figure I.20 : Electron microscope

There are two main types of electron microscopy:

- **Transmission electron microscopy**

  Observation is carried out under ultra-high vacuum using electrons as the radiation source. As the electrons are diffracted as they pass through the sample. The sample is in the form of a very thin section, typically around 50 nm thick. The resulting image appears white against a dark background. The thicker parts of the sample scatter more electrons and therefore appear darker.

- **Scanning electron microscopy**

  Observation takes place in a vacuum. The resulting image is three-dimensional (3D) with a large depth of field. It gives a sense of depth, with light and dark areas.

C-Scanning probe microscopy

The scanning probe microscope has very high resolution and is used to observe very small structures down to the atomic level.

I.6.3 Flow cytometry

Flow cytometry is a technique that allows the rapid analysis of the properties of a large number of particles, often reaching several tens of thousands. analysis provides robust and statistically reliable results.

Principle of operation

Cells suspended in a liquid are directed individually through a laser beam. The physical scattering of the light emitted by the light source is closely linked to certain characteristics such as cell size and granularity.

The laser’s wavelength excites fluorochromes, which then re-emit light across different emission spectra. A single laser can activate up to four or five fluorochromes.

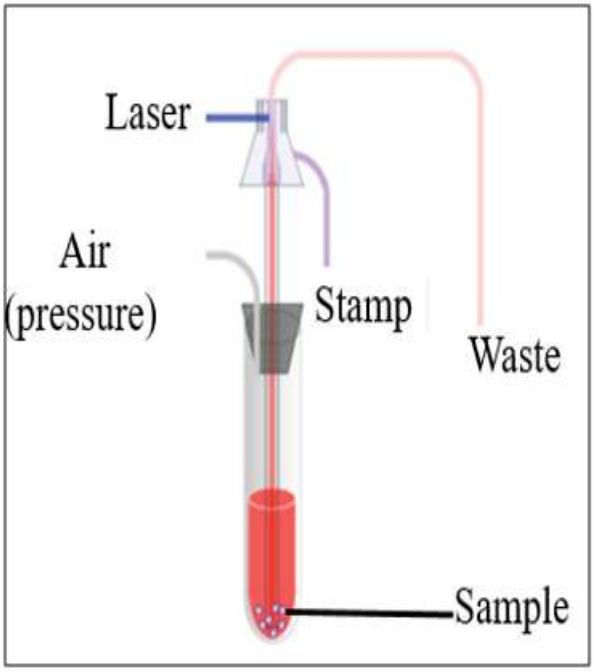


Figure I.21 : principe de fonctionnement de la cytochimie en flux

Graphical representation of the results

The results of flow cytometry are presented as histograms.

- FSM (Forward Scatter) histogram (size) / SSC (Side Scatter) histogram (granularity): Cells are classified by size based on light scattered along the laser axis and by granularity based on light scattered at 90°.

- **Single-parameter histogram**: this provides information on a single parameter for a given population.

- **Biparametric histogram**: this is represented by two signals plotted against one another. Scatter plots are generated, with each point representing a cell.

I.6.4 Selection and isolation

The selection of bacteria allows specific strains to grow on a culture medium known as a selective medium, whilst inhibiting the growth of other strains. Selective media are rich in inhibitors that allow only certain strains to grow. These inhibitors may include antibiotics, salts, dyes…etc.

Isolation techniques

Isolation is a microbiological technique used to separate bacteria in order to obtain clearly distinct colonies from a mixed microbial population. This process is essential for producing pure cultures, which are required for accurate bacteriological analysis or identification. The main objectives of bacterial isolation are:

-To separate the different bacterial species present in a mixed culture.

-To purify the isolated colonies.

-To verify the purity of a bacterial strain grown in either solid or liquid culture media.

-To observe colony morphology, which are clusters of bacterial cells visible to the naked eye.

Bacterial isolation can be carried out either in boxes or in tubes. The technique used for isolation is that of tight striations.

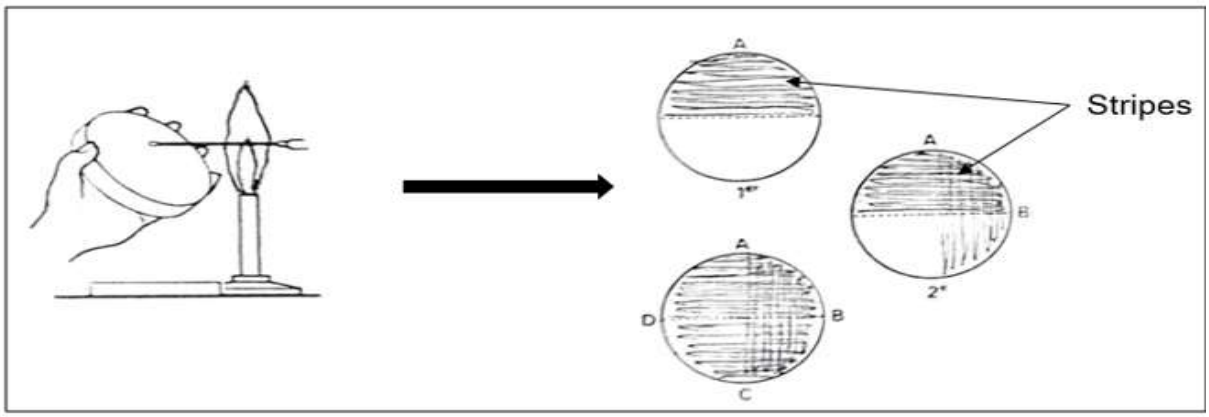


Figure I.22: Method for inoculating a Petri dish

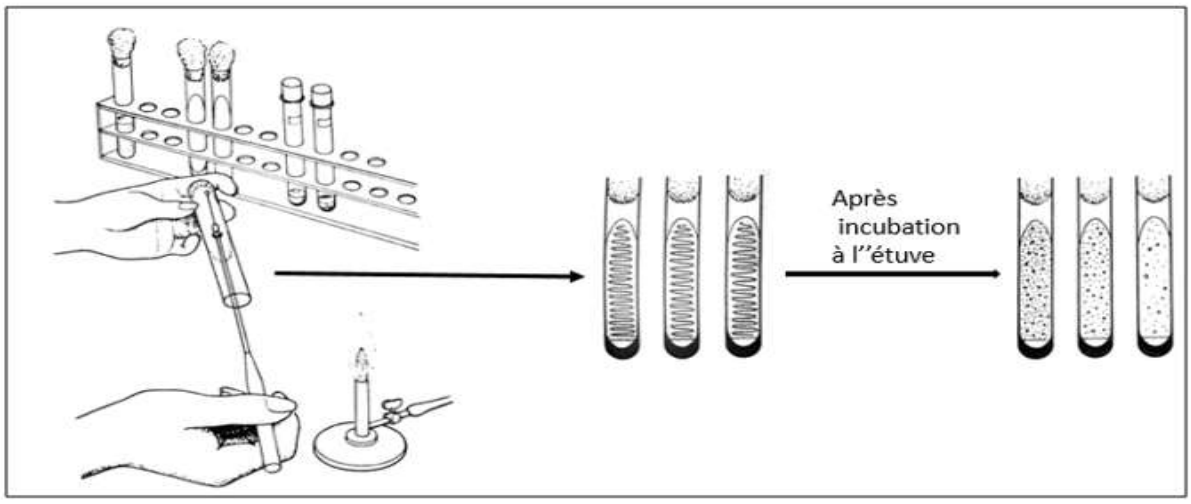


Figure I.23: Method for solid-medium seeding in tubes

Part II :

Environmental biochemistry

II.1 Introduction

Environmental biochemistry is a branch of science that studies the composition, regulation, and analysis of the chemical constituents found in living organisms, including microorganisms, animals, and plants. It also investigates the interactions among these substances, their transformations, the regulation and functioning of these processes, and, consequently, their impact on the physiology of these organisms. This discipline contributes to a better understanding of:

- The function, kinetics of enzymes and their role in environmental biochemical processes.

-The analysis of the microbial degradation of proteins and carbohydrates, and links these processes to the biogeochemical cycles of nitrogen, sulfur, carbon, phosphorus, and oxygen.

-Application of biochemical processes in the environmental field, such as the biodegradation of organic waste and biological remediation.

Biochemistry plays a major role in the environment by helping to understand the interactions between living organisms and the environment. It provides insight into biogeochemical cycles within the biosphere, metabolic and degradation pathways, the involvement of microorganisms in various processes, defenses against pollution, bacteria.

II.2 Molecular components of the cell

The bacterium consists of approximately 80% water and 20% dry matter. The dry matter comprises approximately 90% organic matter and 10% inorganic matter. The bacterial cell is represented by the empirical formula $C_5H_7O_2N$ [18].

The main constituents of bacterial cells include:

• Major elements or macromolecules, namely C, H, N, O, P and S required in large quantities.

• Minor elements or microelements, which are required in small quantities, are Ca, Fe, K, Mg and Na.

• Trace elements, which are supplied in minute quantities, are Co, Mn, Mo, Ni and Zn.

Certain bacteria have specific elemental requirements. For example, bacteria involved in the synthesis of vitamin B_{12} require relatively high levels of cobalt, whereas methane-producing microorganisms depend on sulfur-containing compounds for their metabolic activities. In addition, halophilic bacteria require significant concentrations of sodium (Na) and chloride (Cl) ions to grow and function properly.

Table II.1 : Typical composition of bacterial cells by dry weight

Element	Average composition (%)	Composition range (%)
Carbon	50	45- 55
Oxygen	20	16-22
Nitrogen	14	14- 16
Hydrogen	8	7- 10
Phosphorus	3	2- 5
Sulphide	1	0,8- 1,5
Potassium	1	-
Sodium	1	-
Calcium	0,5	-
Chlorine	0,5	-
Manganese	0,5	-
Iron	0,2	-
Trace element	0,1	-

During the process of anabolism, bacteria synthesise four major types of macromolecules that make up living matter. These macromolecules are:

-**Proteins**: these belong to the family of proteins. They are formed by the linking together of a multitude of basic units, known as amino acids.

-**Polysaccharides:** these belong to the carbohydrate family and are made up of numerous monosaccharide units. Monosaccharides are the simplest carbohydrates, such as fructose, ribose and glucose.

-**Lipids**: These consist of fatty acids and derivatives of complex carbohydrates or sugars.

-**Nucleic acids**: The structure of nucleic acids comprises a sugar (ribose) in RNA and (deoxyribose) in DNA as well as five organic bases (adenine, guanine, cytosine, uracil and thymine.

Basic unit	**Nature of macromolecules**	**The role of macromolecules**
Amino acids →	Proteins	-Structural (cytoplasm, membrane, cell wall) -Functional (enzyme)
Fatty acids →	Lipids	Structural (membrane)
Ose derivatives →	Polyosides	Structural (cell wall)
Dare →	Polyosides	
Organic bases →	Nucleic acids	-Structural (ribosomes) -Functional (DNA that serves as the carrier of the genes controlling protein synthesis)

II.3 Concepts in bioenergetics

Bioenergetics is the study of how energy is generated, utilized, and transferred within cells. These energy transformations occur through the various biochemical reactions taking place in living organisms, collectively known as metabolism, which includes [19, 20]:

• Catabolism is the breakdown of substances to produce ATP.

• Anabolism is the use or storage of ATP for the synthesis of complex organic molecules.

All the reactions depend on the exchange of matter and energy between the environment and the organism.

The study of energy transfers within and between living organisms is called bioenergetics.

The principle of energy balance involves balancing energy intake and expenditure. In humans, energy intake comes from nutritional sources (food). As for energy losses, they are caused by the production of heat and work (mechanical, enzymatic… etc.).

According to the first law of thermodynamics, the change in internal energy ΔU (in joules) of a system depends on the amount of heat ΔQ (in joules) and the amount of work ΔW (in joules) exchanged with its environment:

$$\Delta U = \Delta Q + \Delta W \qquad \text{(Eq. 1)}$$

Bioenergetics studies the supply, use, and transfer of energy within cells. These transfers manifest themselves through the biochemical reactions that occur within an organism: this is metabolism, which includes:

• Catabolism (the breakdown of substances to produce ATP).

• Anabolism (the use or storage of ATP for the synthesis of complex organic molecules).

All the reactions depend on the exchange of matter and energy between the environment and the organism.

The study of energy transfer within and between living organisms is called bioenergetics.

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II.4 Proteins

Proteins are macromolecules formed by the polymerization of approximately 20 different amino acids linked together by peptide bonds. A tripeptide is composed of three amino acids, whereas an oligopeptide contains fewer than 10 amino acids. Chains containing fewer than 100 amino acids are generally referred to as polypeptides, while those consisting of more than 100 amino acids are classified as proteins.

II.4.1 Structure and properties of amino acids

II.4.1.1 Structure

Amino acids are nitrogen-containing characterized by the following general chemical structure:

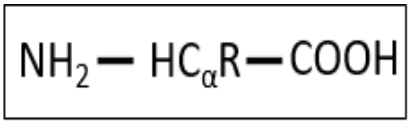


Figure II.1 : Structure of an amino acid

The central carbon (Cα), known as the alpha carbon, is bonded to an amine group (NH_2), to a carboxyl group (-COOH), and to a side chain that varies from one amino acid to another. The first amino acid in the polypeptide chain is the one that carries the N-terminal

amino acid, whilst the amino acid carrying the carboxyl group (the C-terminal amino acid) is the last amino acid.

II.4.1.2 Properties of amino acids

The R groups of amino acids have distinct properties: some are hydrophobic, whilst others are hydrophilic and some are basic in aqueous solutions, whilst others are acidic or neutral.

II.4.2 Structure and properties of proteins

II.4.2.1 Structure

During protein synthesis, amino acids are joined together by peptide bonds within the ribosomes. These bonds are formed through a condensation reaction between the carboxyl group (–COOH) of one amino acid and the amino group ($–NH_2$) of another amino acid, resulting in the formation of a peptide chain [21].

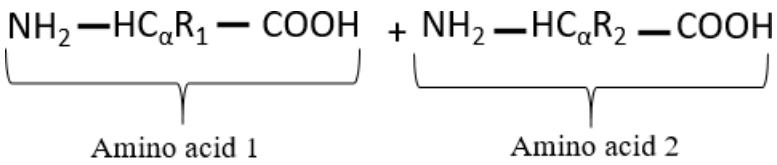


Figure II.2 : Structure of a dipeptide

Depending on their composition, proteins can be classified into several categories.

- **Holoproteins**: these consist solely of amino acids.
- **Heteroproteins:** these are composed of amino acids and a non-protein component known as a prosthetic group, which may consist of lipids, carbohydrates or metal ions.

Depending on their overall shape, we distinguish between:

- **Globular proteins**: which are spherical proteins characterised by their

solubility in water (e.g. haemoglobin, hormones).

- **Fibrous proteins**: These are thread-like proteins that are insoluble in water (e.g. keratin).

- **Mixed proteins**: part globular, part fibrous (e.g. myosin).

II.4.2.2 Properties of proteins

Proteins account for more than half of the dry weight of cells and are involved in all cellular functions (protection, movement, energy, enzymes, nerve impulses, regulation, transport).

A peptide bond is:

- Rigid, as rotation about the bond C—N is impossible.
- Planar, as the Cα, C, O, N, H and Cα atoms lie in the same plane.
- Stable and polar.
- Hydrogen bonds can form, helping to stabilise the secondary structures of proteins.

II.5 Enzymology

II.5.1 Structure and mechanism of action of enzymes

Enzymes are specialised protein-based catalysts accelerating biochemical reactions without altering the outcome [22].

II.5.1.1 Structure of enzymes

Enzymes can be specific proteins composed entirely of α-amino acids (100%), known as holoproteins.

Enzymes known as holoenzymes or heteroproteins are composed of an apoenzyme and a cofactor.

-**Apoenzyme**: a protein component with a primary, secondary, tertiary or

quaternary structure.

-**Cofactor**: a chemical compound essential for enzymatic activity, which may be :

Either an inorganic ion (metal ions) such as Zn^{2+} or Mg^{2+}, which plays a role in stabilising the enzyme's structure and binding the substrate to form an organometallic complex.

These are organic molecules known as coenzymes (usually vitamins) that are essential for enzymatic catalysis. The nature of the coenzyme-enzyme bond allows us to distinguish between two types of coenzymes:

- **Free coenzymes**

  They are so called because they dissociate from the enzyme during each catalytic reaction. In this case, the coenzymes are bound to the enzyme by electrostatic bonds or even weaker interactions. The coenzyme is referred to as the cosubstrate.

- **Bound coenzymes**

  Coenzymes are bound to enzymes by covalent bonds and do not dissociate. The coenzyme is referred to as the prosthetic group.

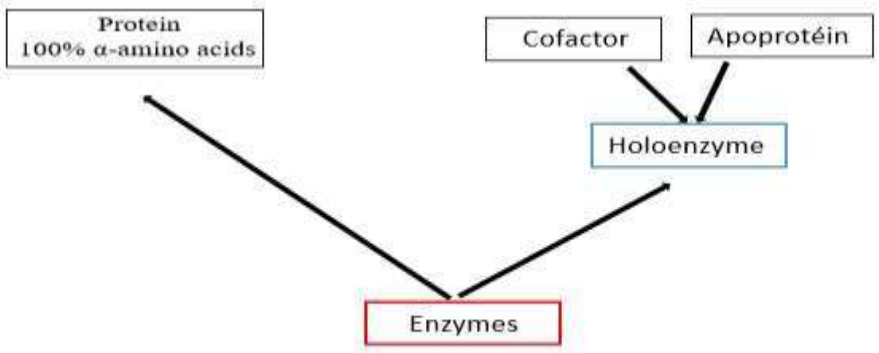


Figure II.3 : Structure of an enzyme

II.5.1.2 Mechanism of action of enzymes

For an enzymatic reaction to occur, the following components are required:

- An enzyme capable of catalysing a specific reaction under appropriate conditions including optimal substrate concentration, temperature, pressure, and pH.

- The substrate, upon which the enzyme acts and which is converted into one or more products during the catalytic process.

- The product: the molecule the molecule generated as a result of the enzymatic reaction catalysed by the enzyme.

- A ligand: a chemical compound that binds specifically to an enzyme through a selective interaction.

The active site, is the specific region of the enzyme where substrate binding and catalysis take place. It consists of two functional parts:

- The substrate recognition site. Composed of specific certain amino acids residues, this region is responsible for recognizing and correctly orienting the substrate thereby determining the enzyme's specificity.
- The catalytic site, formed by specific amino acid residues, is directly involved in the formation and breaking of chemical bonds. These residues are located at the bottom of the active site cavity.

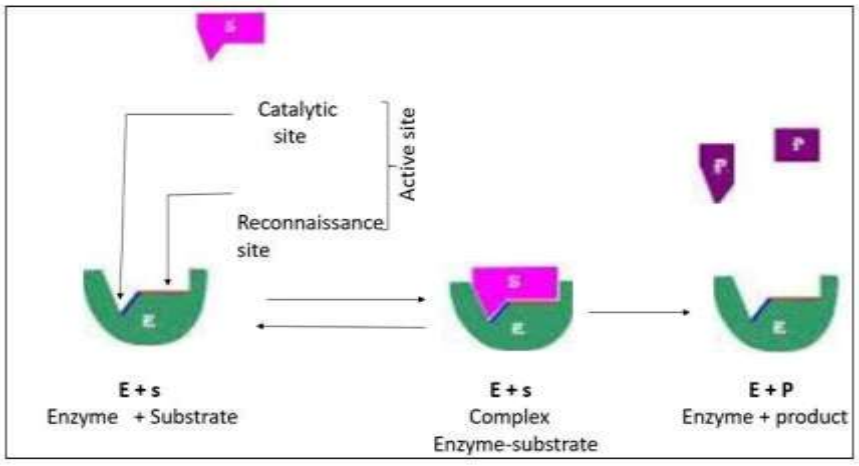


Figure II.4 : Mechanism of action of enzymes

The structure of the active site

Two models have been proposed to explain the specificity of enzymes with regard to their substrates.

-Lock-and-key model: a model proposed by Fischer in 1890, in which the shape of the enzyme's active site is complementary to that of the substrate.

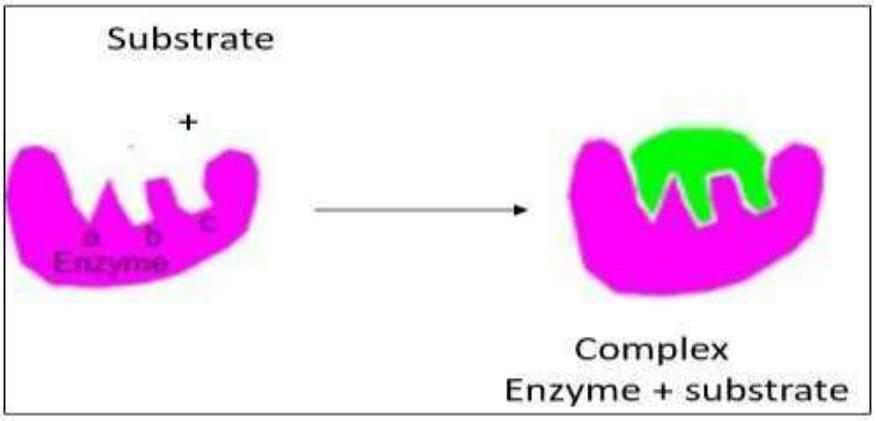


Figure II.5 : Key-and-lock model

-Induced fit: a model proposed by Koshland in 1958. This model suggests that the enzyme and substrate undergo complementary conformational changes upon binding. According to this concept, the enzyme molecule is not rigid but flexible.

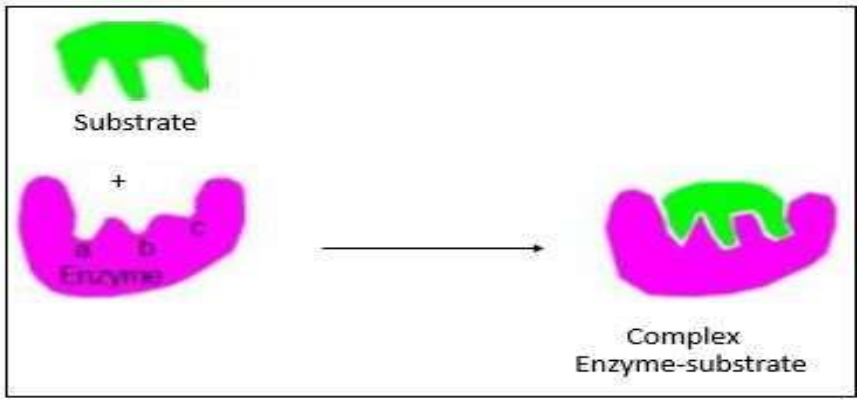


Figure II.6 : Induced shape model

The binding of the substrate to the active site is mediated by several non-covalent interactions, including Van der Waals forces, hydrogen bonds and electrostatic interactions.

II.5.2 Mechanisms of enzymatic catalysis

Enzymatic catalysis is explained by a number of closely related mechanisms:

-Enzymes increase the rate of the metabolic reaction without altering the equilibrium state,

-They lower the activation energy of the substrate,

-They act at very low concentrations,

-They undergo no alteration or modification during the reaction,

-Outside their ideal pH and temperature ranges, their activity decreases or ceases entirely,

-Some enzymes are capable of catalysing a reversible reaction (in both directions),

-They are characterised by broad specificity, where the enzyme can act on a large number of compounds sharing a single structural feature, or by narrow specificity, where the enzyme binds to a single substrate,

-Enzyme activity is regulated by activators, which increase activity, and by inhibitors, which decrease activity.

II.5.3 Further notes on enzyme kinetics

Enzyme kinetics is the study of the rates of enzyme-catalyzed and biochemical reactions catalysed by enzymes. During these reactions, the enzyme (E), acts as a catalyst, converting the substrate (S) into the product (P).

It goes through several phases.

- The pre-steady state phase

When the enzyme (E) comes into contact with the substrate (S), an enzyme–substrate (E–S) complex is rapidly formed.

-The steady-state phase

During this phase, the enzyme becomes with the substrate (S)), and the concentration of the enzyme–substrate (E–S) complex reaches and remains at

its maximum level.

-The post-steady state phase

During this phase, the substrate concentration (S) decreases significantly over time**.**

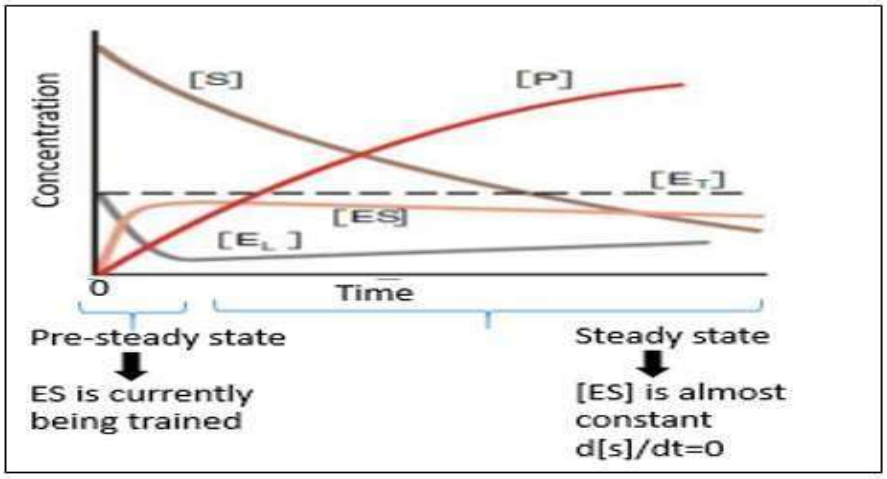


Figure II.7 : The different stages of the enzymatic reaction

The rate of an enzymatic reaction is defined by the change in substrate and product concentrations over time.

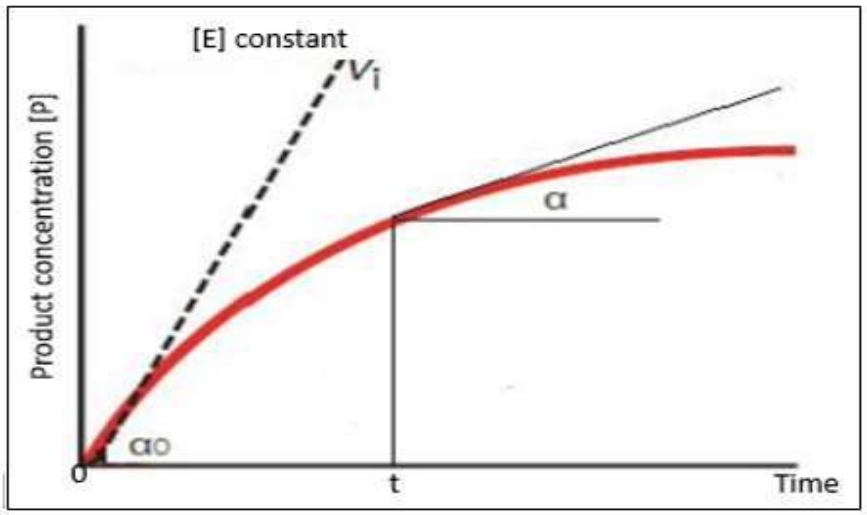


Figure II.8 : Reaction rate

$$V = -\frac{dS}{dt} = \frac{dp}{dt} = K[S] \qquad (Eq\ 2)$$

At time t: V = tan α

At time t_0: V is at its maximum (initial reaction rate), $V_0 = \tan \alpha_0$

-Order of a reaction

La vitesse d'une réaction peut s'écrire suivant la formule suivante :

$$V = K\,[A]^{\alpha}\,[B]^{\beta}\,[C]^{\gamma}\ldots$$

Where:

A, B, C…: are the reactants and products involved in the reaction.

K : rate constant

α, β, γ: are the partial orders of the reaction

n = α + β + γ + …: « n » is the overall order of the chemical reaction

If the reaction is zero-order: the reaction rate is independent of the concentration involved in the reaction process.

$$V = K = -\frac{d[A]}{dt} \qquad (Eq\ 3)$$

If the reaction is of the first order type: the concentration of the reactants decreases exponentially with time.

$$V = -\frac{d[A]}{dt} = K\ [A] \qquad (Eq\ 4)$$

If the reaction is a 2nd order reaction:

$$V_0 = K\ [A]^2 \qquad (Eq\ 5)$$

Enzyme kinetics

The Michaelis–Menten hypothesis:

The basic model of enzyme kinetics is expressed as follows:

Where:

E: enzyme

S: substrate

P: reaction product

Ki: reaction rate constants

The Michaelis Menten constant:

The rate (V) at which the substrate (s) is consumed is equal to the difference between V1 and V2 :

(Eq.6)

According to the mass action law:

$$V_1 = K_1[E][S] \qquad (Eq\ 7)$$

$$V_2 = K_2[ES] \qquad (Eq\ 8)$$

The rate of disappearance of the substrate (S) is equal to the rate of formation of the product (P):

$$V = -\frac{dS}{dt} = \frac{dp}{dt} \qquad (Eq\ 9)$$

V3 =V1 – V2 ⟶ K3. [ES]= K1. [E]. [S]- K2 [ES] (*Eq.10)*

(K2 = K3) [ES]= K1[E]. [S] (*Eq.11*)

$$\frac{K_2 + K_3}{K_1} = \frac{[E][S]}{[ES]} = K_m \qquad (Eq\ 12)$$

K_m is the dissociation constant of the ES complex. It measures the enzyme's specificity for the substrate. When the solution is saturated with substrate, Vi becomes Vmax: the Michaelis Menten equation, the fundamental equation of enzyme kinetics, which describes a hyperbola passing through the origin.

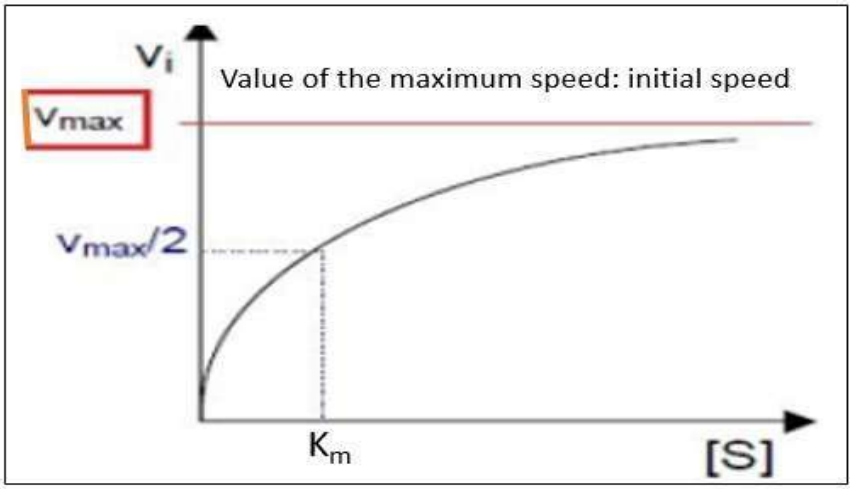


Figure II.9 :Substrate kinetics

II.6 Microbial degradation of proteins

II.6.1 The nitrogen (N_2) cycle

Nitrogen is a key element of nucleic acids and proteins, the major building blocks of living matter. Nucleic acids are responsible for the storage and transmission of hereditary information.The atmosphere, composed of about 79% N_2 is the largest nitrogen reservoir. Although most organisms cannot directly assimilate atmospheric N_2, it enters the nitrogen cycle through several natural processes:

-**The inorganic pathway** : Lightning converts atmospheric nitrogen (N_2) and oxygen (O_2) into nitrogen oxides, while volcanic activity provides an additional natural source of nitrogen to the atmosphere and ecosystems.

- The biological route or symbiosis: the fixation of atmospheric nitrogen by bacteria such as Rhizobium.

The stages of the nitrogen cycle [23]

-Conversion of N_2 into ammonia (NH_3).

-Nitrification: ammonium (NH_4^+) is oxidised into nitrites (NO_2^-) and then into nitrates (NO_3^-).

-Assimilation: plants absorb ammonium and nitrates. These forms of nitrogen are passed on to animals through the food chain.

-Ammonification/decomposition: bacteria and fungi break down dead organisms and waste (urea) into ammonium.

-Denitrification : nitrates are converted into nitrogen gas (N_2) or nitrous oxide (N_2O), releasing nitrogen into the air.

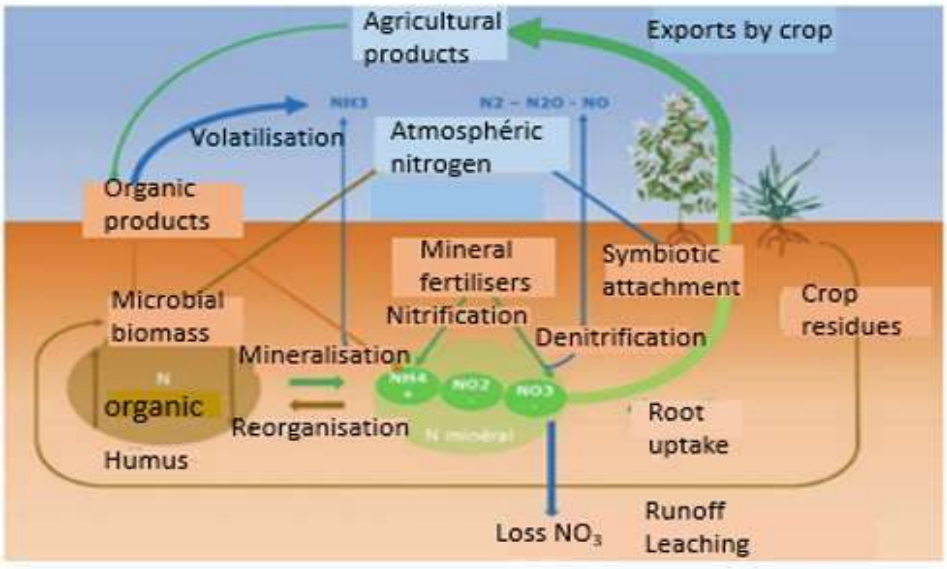


Figure II.10 : Nitrogen cycle[24]

II.6.2 The sulphur cycle

Sulphur is also an essential element for all living organisms because it is a key

constituent of proteins. Autotrophic organisms absorb sulphate ions from their environment and assimilate them into sulphur-containing amino acids, such as cysteine and methionine. These amino acids are essential for protein synthesis, with cysteine contributing to the formation of disulphide bonds that stabilise protein structure.

The organic sulphur synthesised by autotrophic organisms is transferred through all levels of the food chain. Following the decomposition of dead organic matter and excrement, microorganisms mineralise organic sulphur compounds, releasing sulphate ions back into the soil and surrounding environment, where they become available for reuse by living organisms.

-Under aerobic conditions, sulphate ions are formed in water and soil through the oxidation of sulphur. Hydrogen sulphide (H_2S), a toxic gas released from sediments, is oxidised by chemosynthetic bacteria to elemental sulphur or sulphate. The energy released during this oxidation is used by these bacteria to synthesise their own organic matter from inorganic carbon. Photosynthetic microorganisms assimilate carbon dioxide while oxidising hydrogen sulphide (H_2S) to produce Sulphur.

-**In anaerobic** environments such as black mud, sulphate-reducing bacteria reduce sulphate to H_2S. As H_2S rises to oxygen-rich zones, it is oxidised into elemental sulphur or sulphate.

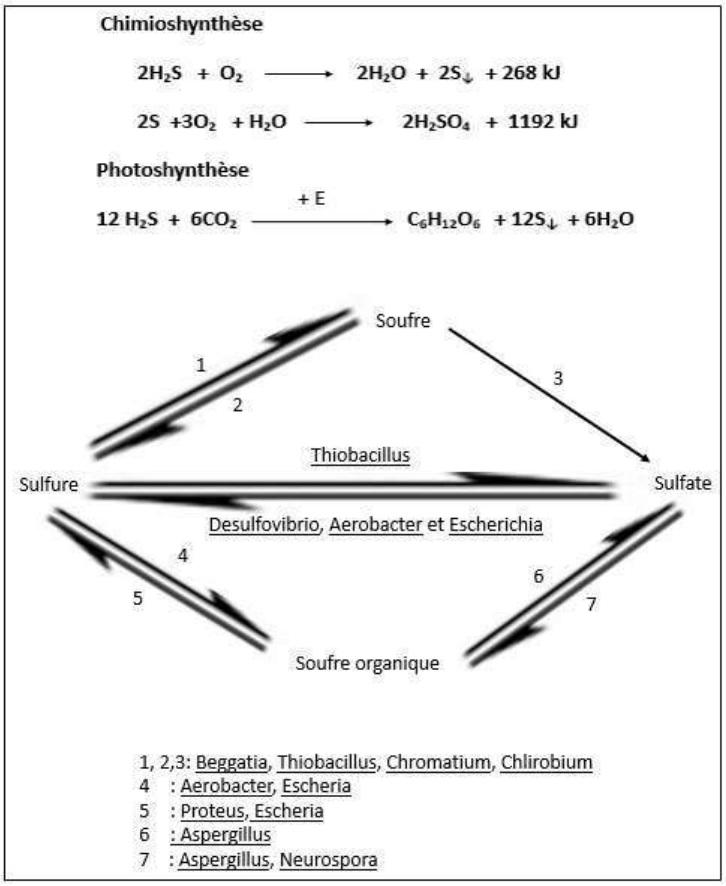


Figure II.11 : Diagram of the chemical reactions involved in the sulphur cycle and the microorganisms that take part in it

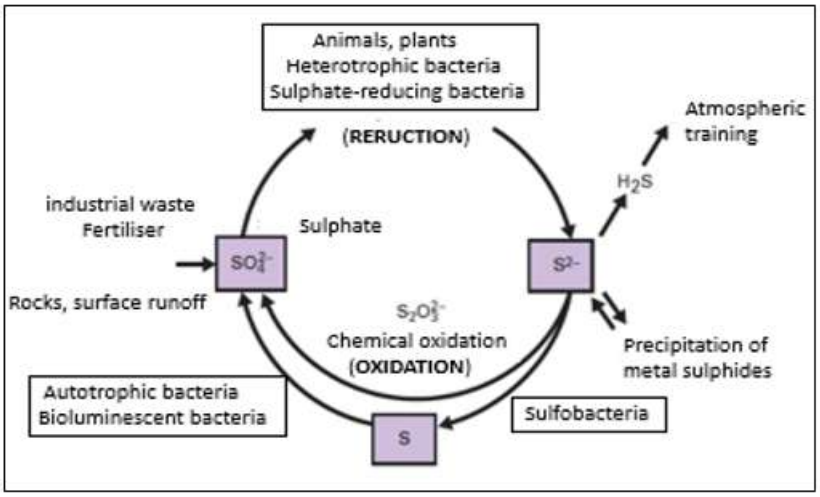


Figure II.12 : Sulphur cycle [25]

II.7 Carbohydrates

Carbohydrates are the most abundant organic compounds in nature. They are produced by green plants, certain bacteria and algae through the process of photosynthesis.

II.7.1 Structure and properties of carbohydrates

II.7.1.1. Structure

Carbohydrates are organic compounds composed primarily of carbon, hydrogen and oxygen, generally following the empirical formula CnH_2nOn. They are characterised by the presence of a carbon chain containing hydroxyl (OH) groups, as well as carbonyl functional group which may be present as either an aldehyde (-CHO) and ketone (C=O) groups. Some carbohydrates or their derivatives may also contain additional functional groups, such as amino ($–NH_2$) or carboxyl (–COOH) groups.

The basic structural unit of carbohydrates is the monosaccharide, which consists of a single sugar molecule containing three (3) to seven (7) carbon atoms such as glucose, ribose, and fructose.

The polymerisation of monosaccharides results in either:

- Oligosaccharides consisting of 2 to 10 monosaccharides, such as sucrose (glucose + fructose).

- Polysaccharides (more than 10 monosaccharides).

- Glycosides composed of one or more monosaccharides linked to an aglycone.

II.7.1.2 Properties

- ✓ Carbohydrates are hydrophilic because their hydroxyl (–OH) groups readily form hydrogen bonds with water molecules.
- ✓ They are reducing sugars because they contain a free aldehyde or ketone functional group.

✓ Components of fundamental molecules such as coenzymes and nucleic acids.

✓ The primary source of energy and energy storage.

II.7.2 Structure and properties of oses

II.7.1.2.1 Structure of ose

Ose (Monosaccharides) are simple carbohydrates that exist in two structural forms: linear (open-chain) and cyclic.

Linear structure

Sugars have the following functional groups:

• (n-1) alcohol groups.

• A carbonyl group, with either an aldehyde group at the end to form aldoses, or a ketone group to form ketoses

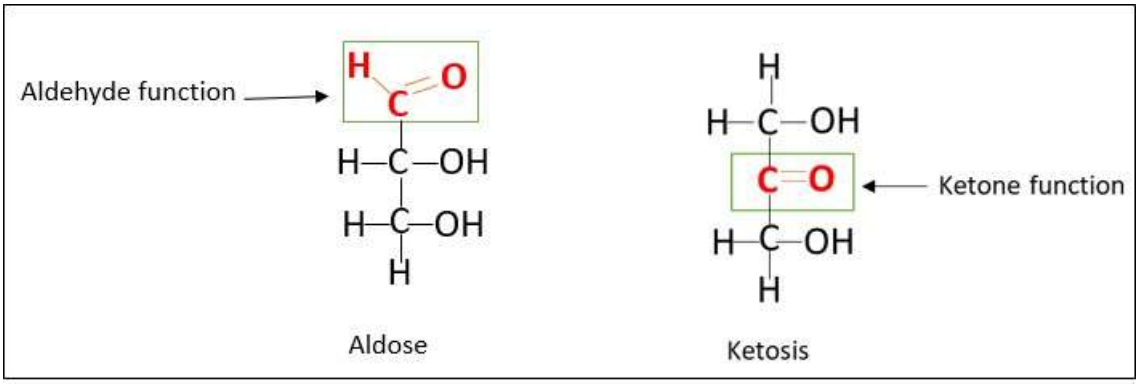


Figure II.13 : Structure of the simplest bones (n=3)

Sugars are classified according to two criteria:

✓ The number of carbon atoms: triose (3), tetrose (4), pentose (5), hexose (6), heptose (7).

✓ The nature of the carbonyl group : aldehyde group (aldose) and ketone group

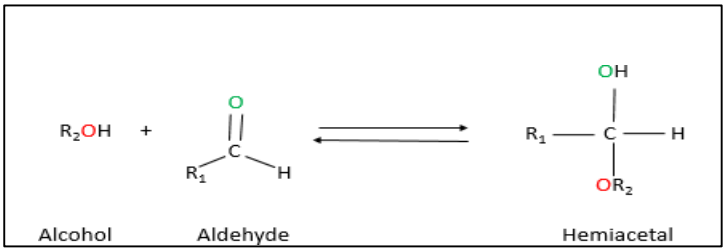
(ketose). Thus, a sugar is classified as.

✓ An aldohexose containing six (6) carbon atoms, five (5) hydroxyl groups and one (1) aldehyde group.

✓ A ketopentose containing five (5) carbon atoms, four (4) hydroxyl groups and one (1) ketone group.

Cyclic structure

Sugars combine with an alcohol molecule to form a hemiacetal, which gives the molecule a cyclic structure. The resulting hemiacetal exists in two forms with different optical rotations: α and β.



The aldehyde group at the C1 position of glucose ($C_6H_{12}O_6$) reacts with the hydroxyl group at the C5 position to form the pyranose ring (a six-membered ring).

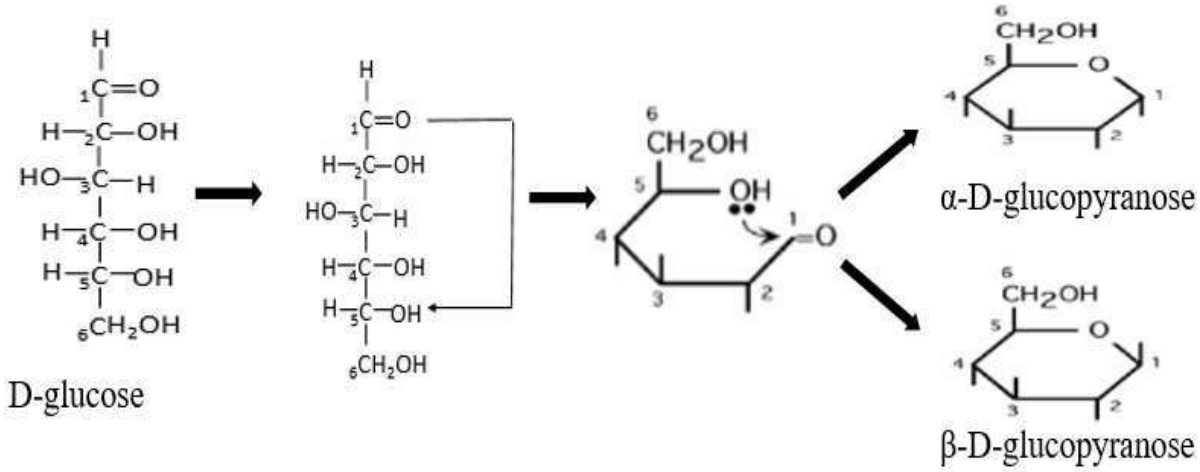


Figure II.14 : Cyclisation of D-glucose to the pyranose form

The furan ring (a five membered ring) is formed by the reaction of the carbonyl group at C2 with the hydroxyl group at C5.

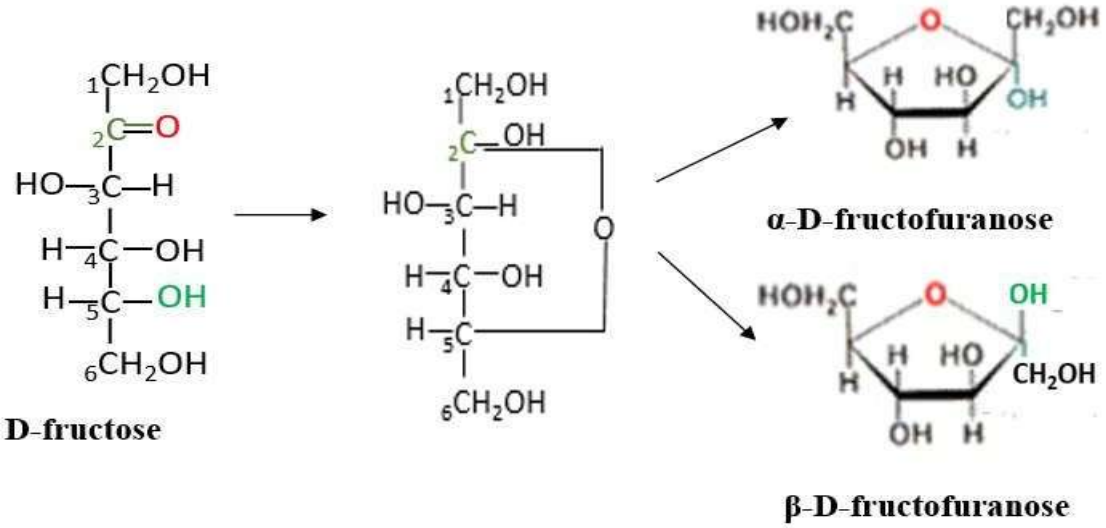


Figure II.15 : Cyclisation of D-frucotse to the furanose form

II.7.2.2 Properties of oses

-The oses are water soluble.

-The oses are soluble in methanol and insoluble in ether.

-They have a thermodegradable structure (caramelization).

-Each oside has a specific rotational power that allows it to be identified.

-Osides do not absorb in ultraviolet (UV) or visible regions of the electromagnetic spectrum. However, characteristic absorption bands in the infrared (IR) region, making infrared spectroscopy a valuable technique for the identification and characterization of sugars.

-.Oses undergo dehydration and cyclization reactions, resulting in the formation of furfural derivatives when heated in a concentrated acidic medium at high temperatures.

-Oses are stable in a dilute acidic medium.

-Ose undergoes complete degradation when heated in an alkaline medium at high temperatures.

-Through chemical processes, aldehyde and ketone groups undergo reduction, resulting in polyols. This reduction is irreversible under chemical conditions but reversible when catalyzed by enzymes.

-They undergo mild oxidation, either chemical or enzymatic conditions. In the presence of stronger oxidizing agents , the sugars undergo more extensive oxidation.

II.8 Microbial degradation of cellulosic waste and the carbon cycle

Cellulose, with the formula $(C_6H_{10}O_5)n$, is a is a linear polysaccharide composed of β(1→4)-linked D-glucopyranose units. It is the main structural component of plant

cell walls, and is also found in sediments and the digestive tract of herbivores animals. It is indigestible by human and most animals, except in ruminants. Many bacterial genera are capable of breaking down cellulose [26, 27].

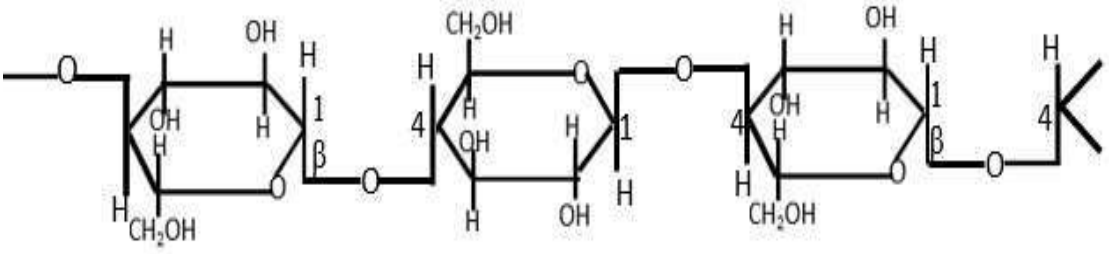


Figure II.16 : structure of cellulose

Numerous microorganisms possess cellulolytic activity, including species of Clostridium, Pseudomonas, and Bacillus. In natural environments, these organisms degrade cellulose by producing a complex of cellulolytic enzymes [28]:

-**Exo-β-1,4 glucanase or cellobiohydrolase**: this enzyme degrades cellulose polymers at the non-reducing ends. It partially degrades cellulose. Its key role is to enable

endocellulase to act on crystalline cellulose.

-**Endo-β-1,4 glucanase or endocellulase**: this enzyme degrades the internal bonds in the cellulose chain. It acts randomly, releasing cellodextrins, cellobiose and glucose. This enzyme is active on soluble celluloses.

-**β-1,4 glucosidase or cellobiase**: it hydrolyses the β-glucosidic bond in cellobiose, releasing two glucose molecules.

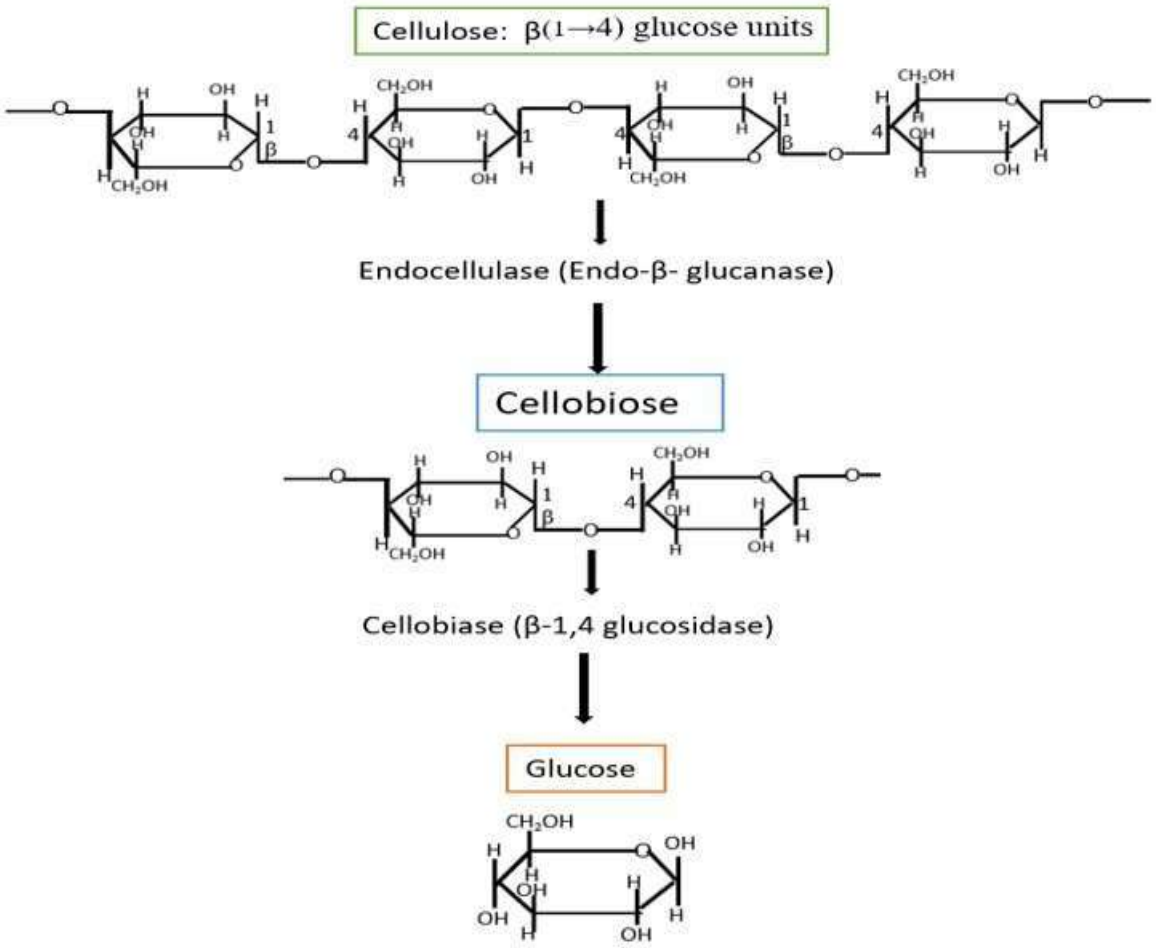


Figure II.17 : Cellulolytic enzymes involved in the breakdown of cellulose

The carbon cycle

The carbon cycle is one of the most sophisticated biogeochemical cycle on Earth. It involves the continuous exchanges of carbon among the atmosphere, biosphere, hydrosphere and lithosphere. Carbon exists in two principal forms: organic carbon (Corg) and inorganic carbon (Cinorg). Organic carbon originates from living organisms including humans, animals and plants, and is incorporated into organic compounds through bonds with It is bound with other elements such as hydrogen (H), phosphorus (P) or nitrogen (N) in organic molecules [29].

Inorganic carbon, in contrast, is bound within inorganic compounds and is not associated with carbon–hydrogen (C–H) or carbon–carbon (C–C) bonds.

Carbon dioxide (CO_2) is converted into organic compounds through reduction during photosynthesis, a process carried out by green plants, certain algae, and photosynthetic bacteria. During this process, these organisms absorb CO_2 from the atmosphere and release oxygen (O_2).

The conversion of the organic carbon obtained into a mineral state is achieved through respiration (release of CO_2 and absorption of O_2).

Organic compounds produced by living organisms return to the soil after they die where they are decomposed by microorganisms. These microorganisms play a crucial role in recycling organic matter and maintaining the carbon cycle. Numerous specialised bacteria (cellulolytic, denitrifying, pectinolytic), both aerobic and anaerobic, are involved in the decomposition of these organic compounds. When the oxidation of organic compounds is incomplete, the carbon skeletons are incorporated into humus or mineralised by specific bacteria. This is a particularly slow process. Humus, meanwhile, is the component responsible for soil fertility. On the seabed and in rivers, mineralisation takes place anaerobically through fermentation.

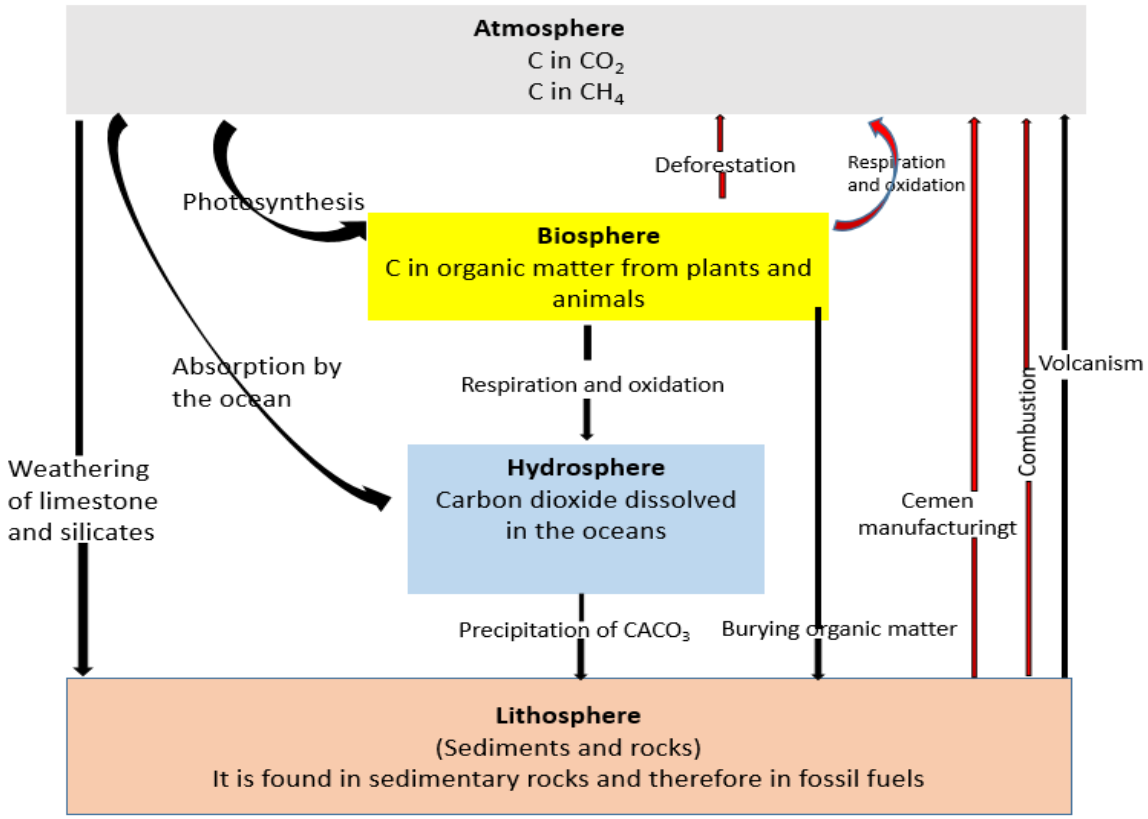


Figure II.18: Carbon cycle [30]

II.9 The phosphorus and oxygen cycles

II.9.1 The phosphorus cycle

Phosphorus is one of the essential elements of living organisms. It is a key constituent of numerous phosphorylated molecules such as phospholipids, nucleotides…etc.

The phosphorus cycle involves interactions between living organisms, the hydrosphere and the lithosphere. However, it does not play a role in atmospheric processes.

Origin

Phosphorus originates from several natural sources, including:

-Erosion: Phosphorus is abundant in rocks of the lithosphere. Under the action of climatic agents such as wind and rainfall, these rocks gradually weather, releasing phosphorus into the environment.

-Uptake by living organisms: Plants absorb phosphate ions from the soil to meet their nutritional requirements for growth and development. Phosphorus is then transferred through the food chain, beginning with plants, which assimilate phosphates, and subsequently passing to herbivores and finally to carnivores through feeding relationships.

-Waste decomposition: When plants and animals die, their organic matter is decomposed by microorganisms. This process releases phosphate ions, which are subsequently returned to the soil and become available for reuse by plants.

-Plankton blooms and sedimentation: Plankton blooms and sedimentation: Phosphates released through rock weathering or excreted by humans, animals, and decomposers are eventually transported to rivers, seas, and oceans. A portion of these phosphates is taken up by plankton, promoting their growth and, in some cases, causing algal or plankton blooms. The remaining phosphates settle on the ocean floor, where they accumulate with sediments. Over millions of years, these sediments are transformed into phosphate-rich rocks, completing the geological phase of the phosphorus cycle.

Human activities can significantly disrupt this natural cycle. The excessive use of phosphate-based fertilizers in agriculture and the discharge of phosphate-rich wastewater into aquatic ecosystems increase phosphorus concentrations in rivers and lakes. This nutrient enrichment promotes eutrophication, leading to excessive algal growth, oxygen depletion, and the degradation of aquatic ecosystems.

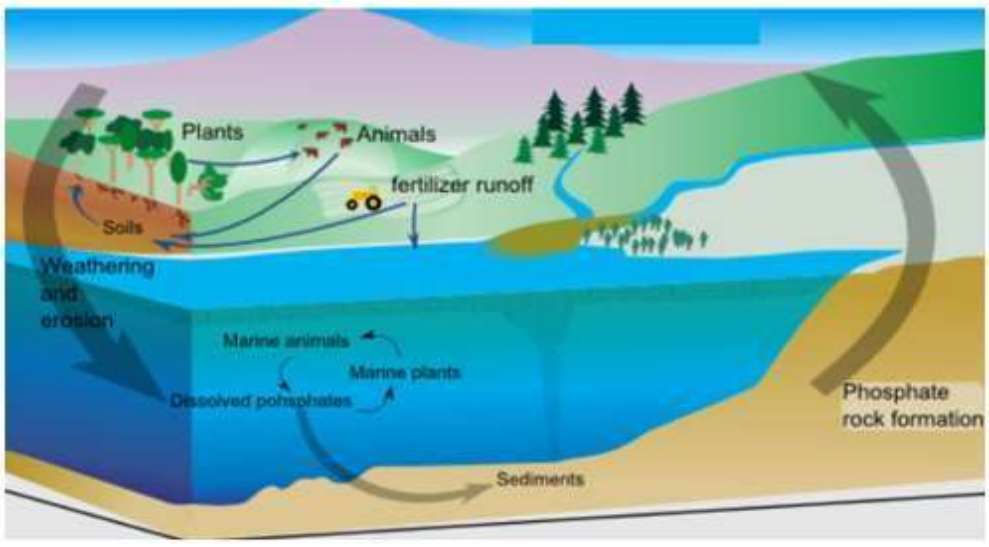


Figure : II.19 : The phosphorus cycle [31]

II.9.2 The oxygen cycle

Oxygen is one of the most important elements for life. The oxygen cycle primarily involves the continuous exchange of oxygen

between living organisms and plants. Humans and animals consume oxygen during respiration, while plants replenish it through photosynthesis, maintaining the balance of oxygen in the environment by consume carbon dioxide, which is itself released by humans and animal.

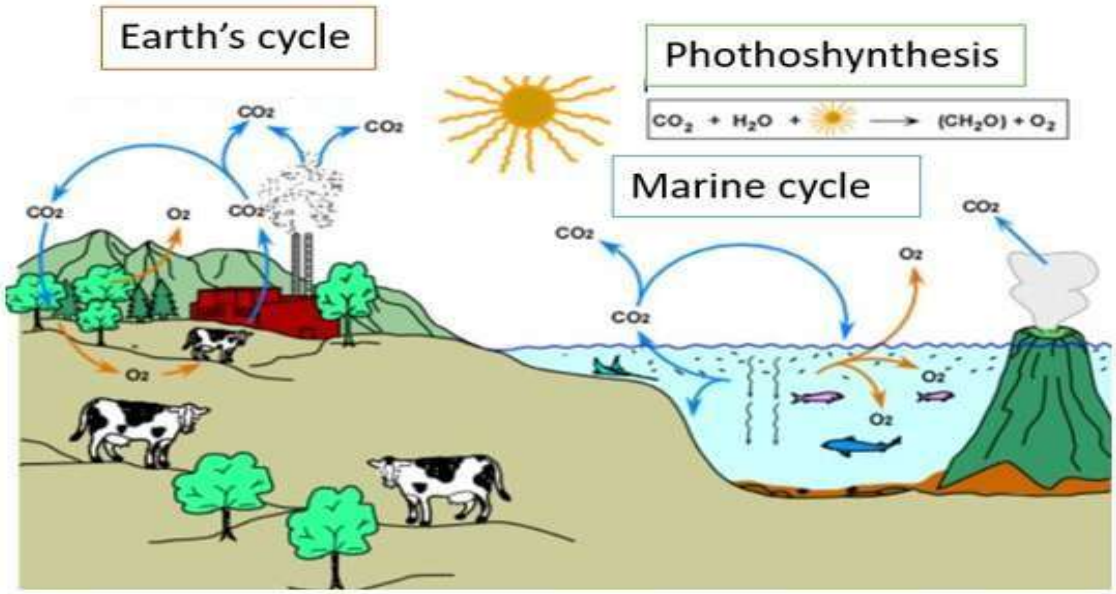


Figure II.20: The oxygen cycle [32]

II.10 Lipids

Lipids are organic molecules composed of carbon, oxygen and hydrogen (C, O, H) with a wide variety of structures. They are characterised by their insolubility in water and their ability to dissolve in non-polar organic solvents.

II.10.1 Structure and properties of fatty acids

II.10.1.1 Structure

A fatty acid is an organic molecule composed of a hydrocarbon chain containing

between 4 and 36 carbon atoms, with the general formula $CH_3–(CH_2)_n–COOH$. They may be saturated, containing no double bonds, or unsaturated, containing one or more double bonds. In some cases, their hydrocarbon chains may also be branched or contain hydroxyl (–OH) groups [33].

The carbon atoms in a fatty acid are identified by numbers: C1, C2, C3, and so on. The C1 carbon atom is part of the carboxyl (–COOH) group, and the last carbon atom carries the methyl ($–CH_3$) group.

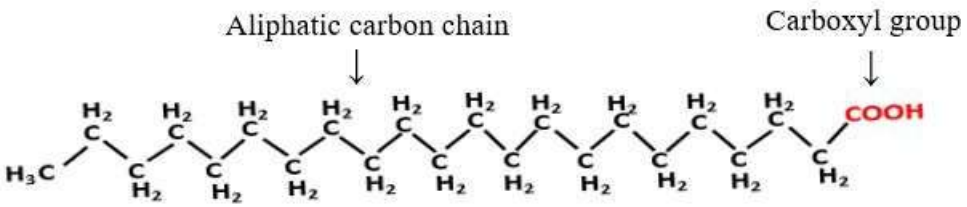


Figure II.21 : Structure of fatty acids

The nomenclature for fatty acids is as follows:

$$Cn : x^{\Delta m,\ n,o}$$

Where:

C : carbon
N : number of carbon atoms x : number of double bonds
Δ : double bond
M, n, o : positions of the double bonds relative to the first carbon atom

Based on the presence or absence of carbon–carbon double bonds, fatty acids are classified into two categories:

a- Saturated fatty acids

These contain no double bonds and have the formula CnH_2nO_2

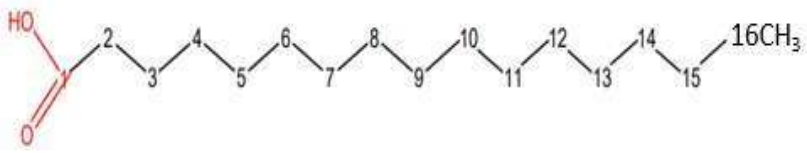


Figure II.22 : Saturated fatty acid (palmitic acid) CH_3-$(CH_2)_{14}$-COOH

b- Unsaturated fatty acids

The presence of a C=C double bond is denoted by the symbol Δ followed by a number of the first carbon atom involved in of the double bond. Unsaturated fatty acids are further classified into monounsaturated fatty acids(MUFAs), and polyunsaturated fatty acids (PUFAs).

- **Monounsaturated fatty acids**: which contain a single double bond.

Example: oleic acid, which has 18 carbon atoms and a double bond between C9 and C10.

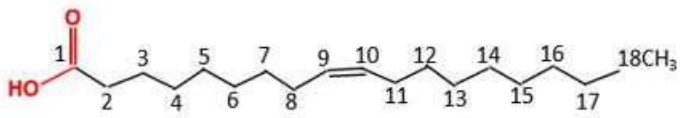


Figure II.23 : Oleic acid (monounsaturated fatty acid), (C_{18}, Δ^9)

- **Polyunsaturated fatty acids**: which contain two or more double bonds.

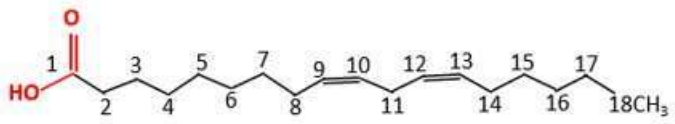


Figure II.24 : Linoleic acid (a polyunsaturated fatty acid), (C_{18}, $\Delta^{9,12}$)

Example: linoleic acid, which has 18 carbon atoms and two non-conjugated double bonds, separated by a methylene group (CH_2).

Fatty acids with multiple double bonds are essential for humans, who cannot synthesize them:

- ✓ **Two double bonds**

Linoléique acid: $\Delta^{9,12}$ C_{18} (F=-5°C): CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH

- ✓ **Three double bonds**

Linolénique acid: $\Delta^{9,12,15}$ C_{18} (F=-11°C): CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH2)_7$-COOH

- ✓ **Four double bonds**

L'acide arachidonique : $\Delta^{5,8,11,14}$ C_{20} (F=-50°C).

c- Hydroxylated fatty acids

These contain one or more hydroxyl groups (OH).

Example: nervonic acid, which has 24 carbon atoms, one OH group and a double bond at C15.

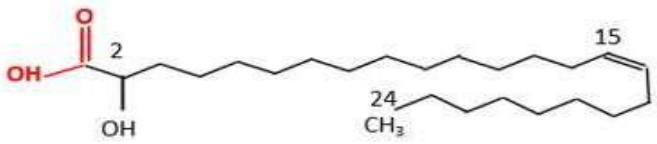


Figure II.25 : Nervonic acid 2 hydroxy C24 :1 Δ^{15}

d- Branched-chain fatty acids

These are generally saturated fatty acids characterised by the presence of one or more methyl groups attached to or incorporated into their carbon chain.

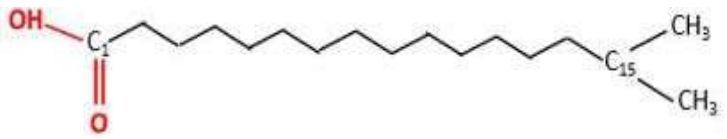
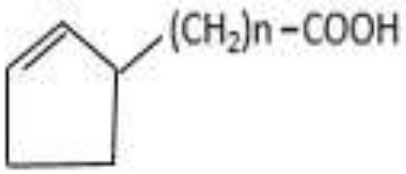


Figure II.26 : 15 méthylhexadecanoic acid

e- Cyclic fatty acids



Hydnocarpinique acid: n=10

Chaulmogrique acid : n=12

II.10.1.2 Properties

-The carboxyl group (-COOH) confers the acidic and hydrophilic properties of fatty acids.

-Their linear hydrocarbon chain is responsible for their hydrophobic nature.

-Saturated fatty acids possess straight, fully hydrogenated hydrocarbon chains, giving their molecules a more elongated and flexible structure.

- Their melting point depends on the number of carbon atoms [34]

E.g. : C12 : 0 → 44°C

C14 : 0 → 53.9°C

- Their melting point depends on their saturation; thus, the melting point is lower if the fatty acid is more saturated

E.g. : C18 :0 → 70°C

-For saturated fatty acids, the boiling point increases with molecular weight. Beyond lauric acid (C12) at normal pressure, decomposition occurs before boiling (palmitic acid, C16, boiling point = 390°C).

- hydrogenation of carbon–carbon double bonds is widely used in the processing of vegetable oils that are rich in unsaturated fatty acids in the food industry.

-Fatty acids are insoluble in water because most of the molecule (the portion

consisting of CH_2) is hydrophobic. Only the carboxyl group is hydrophilic. This property explains the arrangement of lipids in the plasma membrane of microorganisms [34]

II.10.2 Structure and properties of lipids

II.10.2.1 Structure

Lipids are a diverse group of organic compounds composed primarily of carbon, hydrogen, and oxygen. They are insoluble in water but readily dissolve in non-polar organic solvents. Based on their chemical composition, lipids are classified into simple lipids, which contain only carbon, hydrogen, and oxygen (C, H, O), and complex lipids, which also contain additional elements such as nitrogen, phosphorus, or sulfur (C, H, O, N, P, and S).

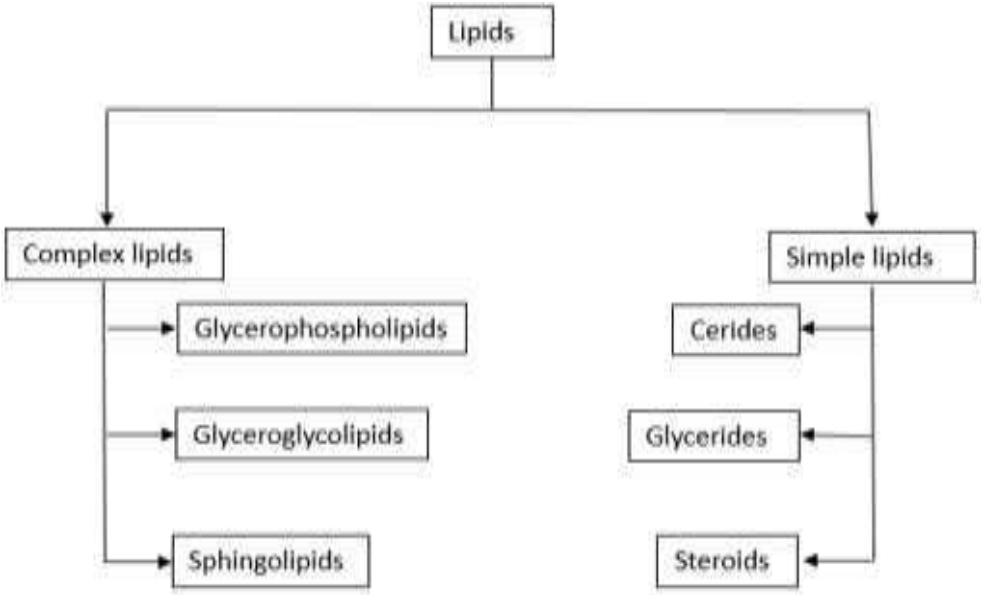


Figure II.27 : Classification of lipids

II.10.2.1.1 Simple lipids

- **Cerides**

These are esters formed by the reaction of fatty acids with alcohols. They are characterised by their long, unbranched hydrocarbon chains containing an even number of carbon atoms.

Due to their non-polar nature, they are hydrophobic, insoluble in water, and generally solid at room temperature.

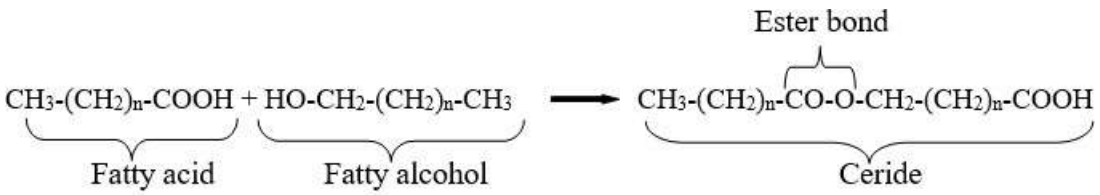


Figure II.28 : Structure of cerides

- **Glycerides**

They are composed of glycerol molecule esterified with one or more fatty acids. The ester bonds are formed through the reaction between the carboxyl (–COOH) groups of the fatty acids and the hydroxyl (–OH) groups of glycerol.

Depending on the number of fatty acids attached to the glycerol backbone, they are classified as monoglycerides, diglycerides, or triglycerides.

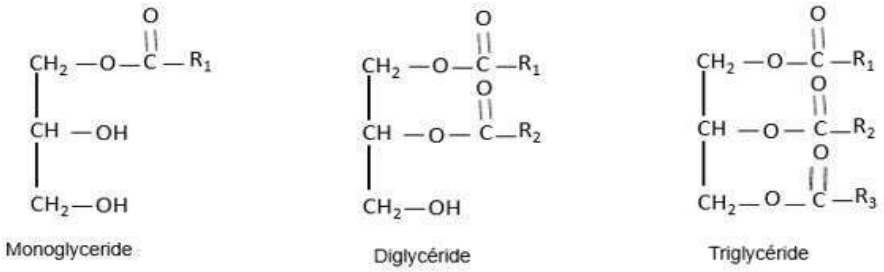


Figure II.29 : The different types of glycerides

- **Sterides**

Sterides are lipids formed by the esterification of a fatty acid with the hydroxyl (–OH) group of a sterol, resulting in esters of sterols and fatty acids.

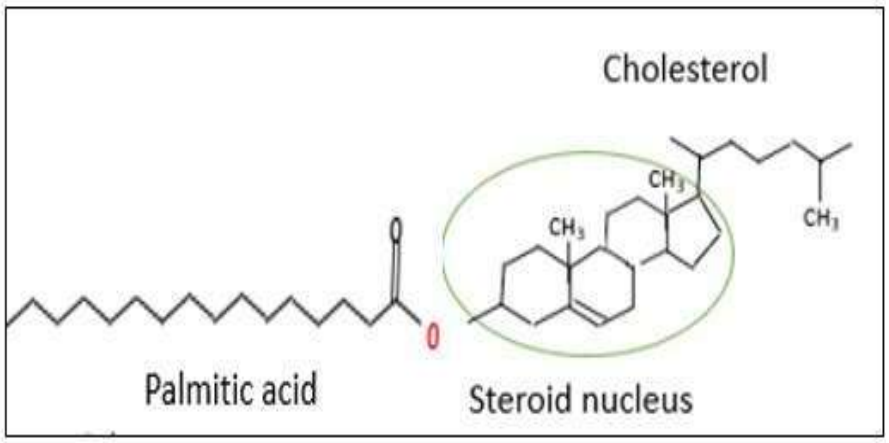


Figure II.30 : Cholesterol pamitate

II.10.2.1.2 Complex lipids

These are heterolipids containing a phosphate, sulphate or carbohydrate group; they are classified as follows:

- **Glycerophospholipids**

They are composed of phosphatidic acid linked to a second alcohol molecule. Phospholipids are amphipathic,possessing a hydrophilic (polar) head and hydrophobic (non-polar) fatty acid tails

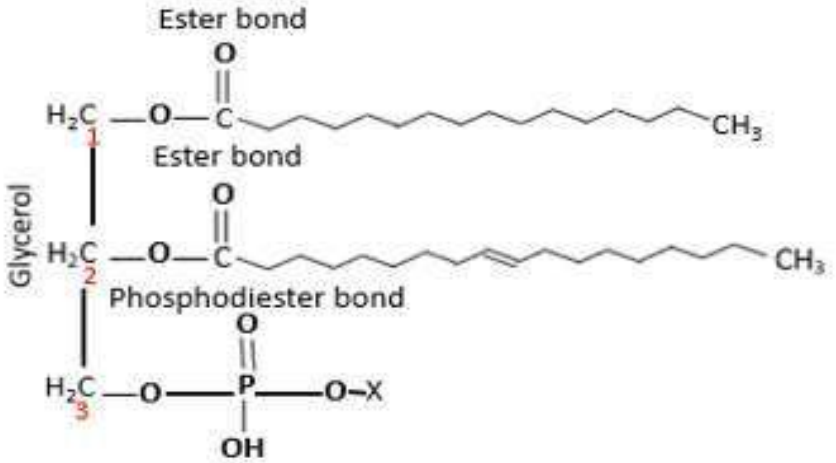


Figure II.31 : Structure of glycerophospholipid

- **Glyceroglycolipids**

They are formed by the esterification of glycerol with one to three fatty acids. The hydroxyl group on the third carbon 3 (C3) is linked via a glycosidic bond to a sugar; therefore, it is not esterified.

- **Sphingolipids**

They are derived from sphingosine, which is an amino-dialcohol group that does not contain glycerol. They are formed by the binding of the carboxyl group of the fatty acid to the NH_2 group of sphingosine

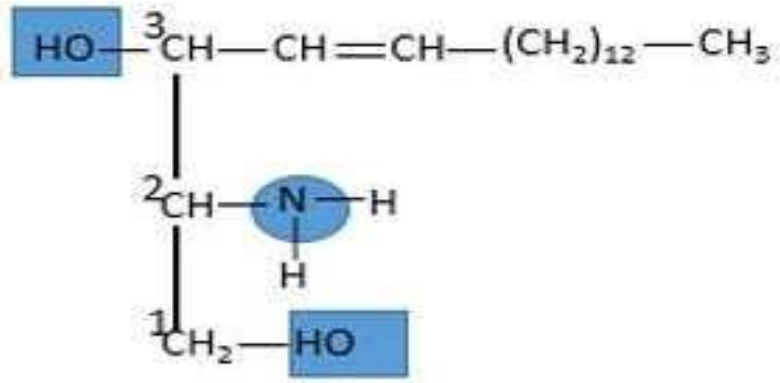


Figure II.32 : Structure of Sphingosine

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II.10.2.1.3 Unsaponifiable lipids

Unsaponifiable lipids account for 0.3 to 2% of total lipids. These are lipids that cannot be hydrolyzed in a basic environment.

All of these compounds have a common precursor: “isoprene (5C).”

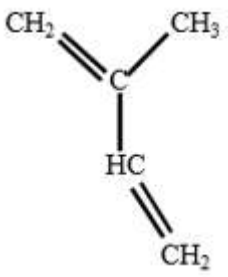

Table II 2: Classes of compounds derived from the precursor (isoprene)

Terpene	Number of carbon atoms	Example
Diterpenes	20	a-Tocophérol : vitamine E
Triterpenes	30	Steroidal derivatives
Tetraterpenes	40	Carotenoids
Polyisoprene	>40	

Note :

There are other unsaponifiable lipids, such as hydrocarbons.

II.10.2.2 Properties of Lipids

-Lipids are hydrophobic molecules (insoluble in water).

-They are highly soluble in the most nonpolar solvents, such as acetone.

-They form highly unstable emulsions that break down into a two-phase system; surfactants disperse them.

-At room temperature and depending on the degree of acid saturation, lipids exist in a liquid (oil) or solid (paste-like) state.

During chemical hydrolysis, acid treatment releases fatty acids and glycerol.

II.11 Microbial degradation of petroleum residues, such as n-alkanes

Hydrocarbons are organic compounds consisting solely of carbon and hydrogen and are highly insoluble in water. Low-molecular-weight hydrocarbons are gaseous, while those with higher molecular weights are liquid or solid at room temperature. Carbon and hydrogen can form saturated, linear, or cyclic molecules. Hydrocarbons include various petroleum products such as crude oil, refined petroleum, kerosene, gasoline, fuel oil, lubricants, and motor oils.

Biodegradation is the process by which microorganisms (bacteria, fungi) alter the chemical structure of a compound into natural metabolic products, resulting in its conversion into water (H_2O), carbon dioxide (CO_2), and/or methane (CH_4), as well as cellular biomass.

Hydrocarbons released into the environment undergo a series of transformations that, in the short or long term, lead to their total or partial disappearance. Microbial transformations carried out by microorganisms are responsible for the breakdown of most hydrocarbons, since these microorganisms—primarily bacteria—use hydrocarbons as substrates for growth. Does each microorganism utilize a specific hydrocarbon in a specific environment? Each species has specific requirements: chain length, branching, and establishment.

Many fungal genera, including *Talaromyces*, *Aspergillus*, and *Fusarium*, are capable of degrading hydrocarbons. However, they are rarely used as a source of carbon. Ligninolytic fungi (wood-decomposing fungi or white rot fungi) can degrade PAHs using enzymes such as lignin peroxidases, manganese-dependent peroxidases, phenol oxidases, or epoxide hydrolases.

There are several pathways for the biodegradation of petroleum hydrocarbons, such as mono- terminal oxidation, sub-terminal oxidation, ω-oxidation and β-oxidation [35, 36, 37].

The degradation of alkanes occurs via mono-terminal oxidation. Oxidation begins at the terminal methyl group, with the formation of a primary alcohol through the introduction of molecular oxygen. The primary alcohol thus formed is oxidised to an aldehyde and then to a fatty acid. Next comes the β-oxidation stage, characterised by the formation and removal of acetyl coenzyme A, through which the fatty acid is broken down into two-carbon compounds. In contrast, in the di-terminal pathway, the oxidation of both ends of the alkane molecule is carried out by γ-hydroxylation of the fatty acids, followed by conversion to a dicarboxylic acid and, finally, by β-oxidation [38, 39].

The final pathway, known as sub-terminal oxidation, involves the oxidation of alkanes to form a secondary alcohol, which is subsequently converted into a ketone and a corresponding ester. The ester is hydrolysed to produce an alcohol and a fatty acid, which can then enter further metabolic degradation pathways.

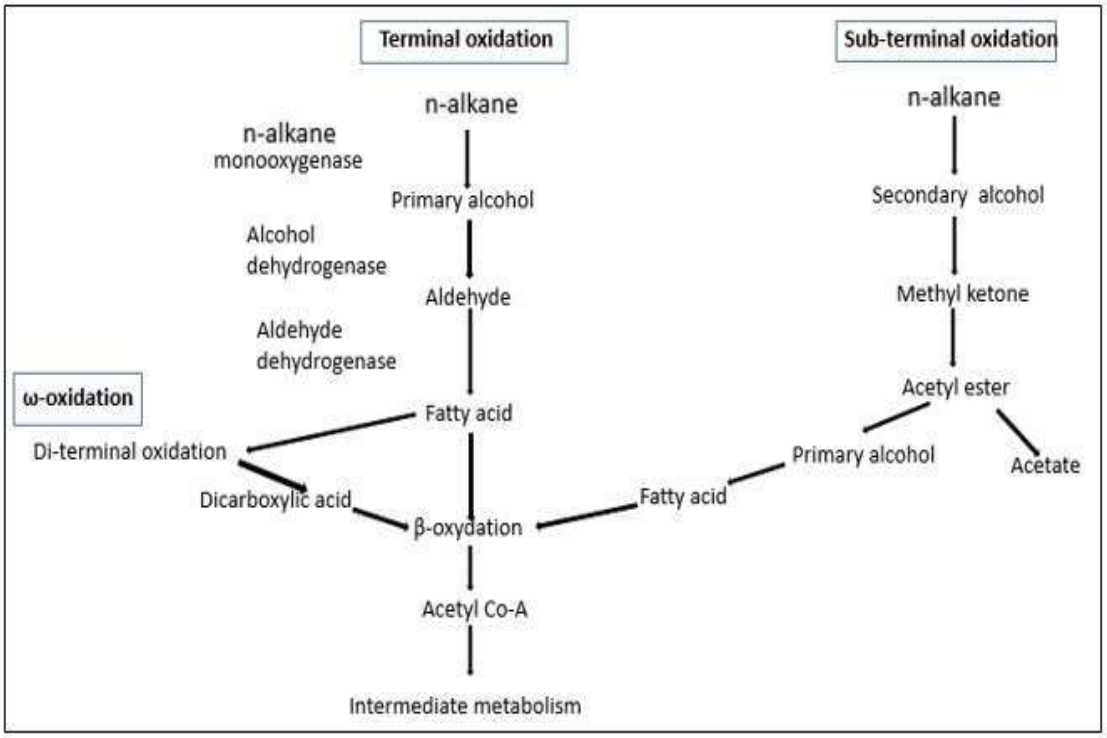

Figure II.33 : Possible pathways for the aerobic degradation of n-alkanes by microorganisms

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Programme de la matière

. Semestre: 1

Unité d'enseignement: UED 1.1

Matière au choix 1: Microbiologie et biochimie de l'environnement

VHS: 22h30 (Cours: 1h30)

Crédits: 1

Coefficient:1

Objectifs de l'enseignement :

Acquérir les connaissances fondamentales de microbiologie de l'environnement.

Connaissances préalables recommandées :

Notions de base de sciences naturelles

Contenu de la matière :

I-Introduction à la microbiologie de l'environnement

II-Morphologie et anatomie fonctionnelle des bactéries

III-Physiologie bactérienne

a)-nutrition

b)-croissance

IV-Rôle des micro-organismes dans le cycle des bioéléments

a)-Caractéristiques des écosystèmes microbiens.

b) Interactions interspécifiques

c)-Microbiologie du sol

d)-Microbiologie des milieux aquatiques.

e)-Microbiologie de l'air.

V-Microbiologie des eaux domestiques et des eaux usées.

VI- Etude de la biodiversité microbienne

1. Echantillonnage
2. Microscopie
3. Cytométrie de flux
4. Sélection et isolements
5. Méthodes Moléculaires

6. Autres Méthodes

Partie biochimie

I- Introduction

a)-Constituants moléculaires de la cellule.

b)-Notions de bioénergétique.

II- Les protéines

a)-Structure et propriétés des acides aminés.

b)-Structure et propriétés des protéines.

III- Enzymologie

a)-Structure et mécanisme d'action des enzymes

b)-Compléments de cinétique enzymatique

IV- Dégradation microbienne des protéines

Cycle de l'azote et du soufre

V-Les glucides

a)-Structure et propriétés des oses.

b)-Structure et propriétés des glucides

c)-Dégradation microbienne des déchets cellulosiques et cycle du carbone.

d)-Le transport d'électrons et cycle du phosphore, de l'oxygène.

VI-Les lipides

a)-Structure et propriétés des acides gras.

b)-Structure et propriétés des lipides.

c)-Dégradation microbienne des résidus pétroliers, les n-alcanes par exemple

Mode d'évaluation : Examen : 100%.

Références bibliographiques: (Si possible)

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