Course content

- Morphology and structure of the bacterial cell
- Bacterial nutrition and physiology
- Bacterial growth

Course Objectives
By the end of this course, the student will be able to:

- Understand the types of morphologies and the elements of the structure of a bacterial cell (essential elements, non-essential elements);
- Identify the nutritional requirements and the physicochemical conditions necessary for the survival of a bacterial cell;
- Understand the concept of bacterial growth and recognizing the parameters that influence the growth kinetics.

The bacterial cell

The microscopic world is predominantly prokaryotic, consisting of archaea and bacteria. Most of the known and studied prokaryotes belong to the domain of bacteria, which are characterized by their immense diversity and the various environments they inhabit[1].

Bacteria are highly adapted to their environments and interact with them. However, the thousands of bacterial species discovered so far distinguish themselves from each other based on a multitude of factors, including morphological ones (size, cell shape), chemical ones (chemical composition of the cell wall), physiological ones (nutrient requirements, environmental factor needs), biochemical ones (types of biochemical activities and required energy source), and genetic ones (genome structure, number and nature of genes).

1. Morphology

1.1. Size

The size of bacteria typically falls between that of large viruses and that of unicellular eukaryotes (single-celled algae, protozoa). However, the average dimensions of most bacteria fall within narrower ranges (0.2 to 2 µm in diameter and 1 to 10 µm in length). For example, enterobacteria like *Escherichia coli* have dimensions of 1 µm × 2 to 4 µm. The small size of bacteria, compared to eukaryotic cells, is an important factor that promotes their growth and multiplication, enabling them to quickly adapt to environmental conditions and easily exploit resources in their environment.

1.2. Cell shape and arrangements

There is a wide variety of shapes among bacteria, but three shapes are the most common: spherical/ovoid shape or cocci, cylindrical shape or bacilli, and spiral shape. In addition to the three main shapes, other forms can also be found, such as star-shaped cells (e.g. genus *Stella*), filamentous bacteria (iron bacteria), as well as branching or non-branching mycelial forms (actinomycetes).

When they divide to reproduce (through binary fission), bacteria can remain attached to each other and form characteristic groupings of the species to which they belong. The planes along which bacteria divide

[1] A large number of bacterial species are ubiquitously distributed, meaning they are capable of growing in a wide range of environments (water, soil, air, eukaryotic hosts, etc.).

determine what is known as the grouping mode of cells. Bacteria that do not remain attached to each other after division have isolated or random arrangements.

❖ **Ovoid shape (coccus)**

Cocci are usually round but can be oval, elongated, or flat on one side (kidney-shaped). The arrangements of cocci can vary. Thus, cocci that remain grouped in pairs after dividing are called diplococci (*Enterococcus faecalis*); those that form chains of varying lengths are called streptococci (*Streptococcus thermophilus*); those that divide along two planes and form groups of four cells are referred to as tetrads (genus *Pediococcus*). Those that divide along three planes and remain attached in cubic groups of eight cells are known as sarcinae (genus *Sarcina*). Those that divide along a multitude of planes and form clusters are called staphylococci (*Staphylococcus aureus*).

❖ **Rod Shape**

Most bacilli are simple rods. Some bacilli resemble straws or have tapered ends. Others are oval at the ends and have an appearance so similar to cocci that they are called coccobacilli (*Escherichia coli*, *Salmonella*). Bacilli can also form associations. However, there are fewer groupings of bacilli observed compared to cocci. Diplobacilli (*Moraxella*) remain attached in pairs after division, and streptobacilli (*Lactobacillus bulgaricus*) form chains.

❖ **Spiral Shape**

Spiral bacteria have one or more curves; they are never straight. Those with a curved rod shape resembling commas are called vibrios (*Vibrio cholerae*). Others, known as spirilla (*Campylobacter jejuni*), have a helical shape and a relatively rigid body. A third group is characterized by a longer, flexible helical shape; these are the spirochetes (*Treponema pallidum*).

2. Structure

Electron microscopy has allowed for a better study of the structure of the bacterial cell. Thus, the bacterial cell is surrounded by a rigid envelope called the cell wall, which gives it its shape and resistance and encloses another much thinner and more fragile envelope, the cytoplasmic membrane. The underlying cytoplasm is homogeneous, containing ribosomes and reserve substrates (lipid reserves, glycogen, phosphate, sulfur, etc.) in the form of granules, but most importantly, the nuclear apparatus or bacterial chromosome, which is characterized by its finely reticulated fibrillar appearance. These structures are essential to the cell and are always present, while other secondary, variable structures may be present: the capsule, an outer envelope present when the conditions for its synthesis are met, flagella responsible for bacterial mobility, pili and fimbriae, appendages finer than flagella, plasmids, small non-circular chromosomal DNA, and spores, a form of resistance to extreme physicochemical conditions found in some species only.

2.1. Cell wall

The wall is a characteristic envelope of the bacterial cell. It is a relatively rigid exoskeleton, of a polymeric nature. A basic polymer specific to bacteria, called peptidoglycan, is present in all bacterial walls.

However, the structure and chemical composition of the wall are not the same for all bacteria. Based on this difference, bacterial species are divided into two major groups, Gram-positive bacteria and Gram-negative bacteria. Gram staining reveals these differences in a straightforward manner.

In electron microscopy, a notable difference in the structure of the walls of Gram-positive and Gram-negative bacteria can be observed. The wall of Gram-positive bacteria is generally thicker (20 to 80 nm) and appears more homogeneous, while that of Gram-negative bacteria is more heterogeneous and thinner (6 to 15 nm).

2.1.1. Functions

Regardless of its structure and chemical nature, the cell wall plays a significant role in:

- The cell's shape.
- Resistance to the high internal pressure (between 2 and 20 atmospheres) of the cell (the cytoplasm is a hypertonic environment).
- Some layers of the cell wall serve as major antigenic determinants.

2.1.2. Peptidoglycan

The cellular shape and wall rigidity are primarily due to peptidoglycan (also called murein or mucocomplex), which is present either alone or in association with other substances. This polymer is composed of a meshwork of three different elements:

- A backbone consisting of alternating glucosidic molecules: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) (this is the glycan chain).
- A set of identical peptidic side chains, composed of 4 amino acids and attached to N-acetylmuramic acid.
- A set of identical "interpeptidic bridges."

The glycan chains are the same for all bacterial species, while the tetrapeptide side chains and interpeptidic bridges vary from one species to another.

2.1.2. Gram-positive bacteria cell walls

In most Gram-positive bacteria, the cell wall is composed of multiple layers of peptidoglycan (which can exceed 25 layers or sheets), forming a homogeneous, thick, and rigid structure. In addition to peptidoglycan, the cell wall of Gram-positive bacteria contains teichoic acids, which are primarily composed of an alcohol (glycerol, ribitol) and phosphate. There are two classes of teichoic acids: lipoteichoic acid, which traverses the layers of peptidoglycan and binds to lipids in the plasma membrane, and teichoic acid, which attaches to the outer and inner layers of peptidoglycan only. Teichoic acids may play a role in regulating cell flow (entry/exit), protecting against wall lysis, and also exhibit antigenic properties.

2.1.3. Gram-negative bacteria cell walls

The wall of Gram-negative bacteria contains a thin layer of peptidoglycan only, outside of which is a double phospholipid layer called the outer membrane. This membrane contains lipoproteins that anchor it to the peptidoglycan and provide some structural integrity to the whole structure.

The outer membrane also contains lipopolysaccharides (LPS), which replace phospholipid molecules in certain areas. The carbohydrate portion is composed of sugars known as O polysaccharides (O antigen), which function as antigens. Meanwhile, the lipid portion is called lipopolysaccharide A or endotoxin. Upon cell death, this endotoxin is released and becomes toxic when it enters the bloodstream of animals.

Lastly, membrane proteins are clustered to form pores in the outer membrane, known as porins. They allow the passage of small molecules such as nucleotides, disaccharides, peptides, amino acids, vitamin B12, iron, etc.

The space between the inner surface of the outer membrane and the cytoplasmic membrane is called the periplasmic space and contains the periplasm. The periplasmic space has a high concentration of degradation enzymes and protein transporters and houses the peptidoglycan layer.

2.2. Cytoplasmic membrane

The plasma membrane, also known as the cytoplasmic membrane or inner membrane, is a thin structure (approximately 8 nm thick), both flexible and sturdy, that lies beneath the cell wall and surrounds and contains the cell's cytoplasm. This vital structure serves as a barrier separating the interior of the cell (cytoplasm) from its environment.

2.2.1. Structure

In bacteria, the cytoplasmic membrane is primarily composed of phospholipids arranged in a double layer, penetrated by membrane proteins. Bacterial cytoplasmic membranes are less rigid than those of eukaryotes because they lack sterols. The biomolecular structure of the cytoplasmic membrane is not static; it adheres to the fluid mosaic model, where molecules can move by exchanging their positions at very high frequency.

❖ **Phospholipids**

Phospholipid molecules form two parallel rows or a double layer. Each phospholipid molecule consists of a polar head, composed of a phosphate group and glycerol (in the form of glycerol phosphate), which is hydrophilic, and a nonpolar tail composed of fatty acid chains that are hydrophobic. The polar heads occupy both exposed surfaces of the phospholipid bilayer, while the nonpolar tails are oriented toward the interior of the bilayer.

❖ **Membrane Proteins**

Protein molecules can be arranged in several ways within the membrane. Some, called peripheral proteins, are located on the inner or outer surface of the membrane and are often enzymes or structural molecules. Other proteins, known as intrinsic proteins (transmembrane proteins), embed themselves in the phospholipid bilayer by spanning it completely and often play a role in membrane transport.

2.2.2. Functions

i. Highly selective permeability barrier, allowing the bacterium to concentrate specific metabolites and excrete its metabolic waste products.

ii. Substance transfer: The cytoplasmic membrane is permeable to water and many molecules, selectively allowing the passage of certain small organic and mineral molecules while preventing the passage of macromolecular compounds.

- **Osmosis**

Water diffuses through the cytoplasmic membrane by the process of osmosis.

- **Simple diffusion**

For small hydrophobic molecules (N_2, O_2, etc.) or non-polar, uncharged molecules (urea, CO_2, etc.), molecular flux occurs along the concentration gradient of the solute. This is a form of simple passive diffusion.

For larger hydrophilic molecules, diffusion along the concentration gradient can occur in two ways:

- Facilitated diffusion through carrier proteins (transporters), involving a conformational change in the protein during transport.
- Facilitated diffusion through protein channels, without a change in structure (higher transfer rates).

- **Active transport**

Bacteria selectively admit and concentrate certain substances while excluding others. This allows them to maintain chemiosmotic balance across the membrane and transport substances against a concentration gradient. In all cases, transfer mechanisms require an input of energy and fall under the term "active transport." Several systems exist in bacteria for this type of transport: i. primary active transport or uniport; ii. group translocation transport (involving phosphorylation of phosphoenolpyruvate (PEP)); and iii. secondary active transport (cotransport: symport and antiport).

iii. Export of proteins synthesized in the cytoplasm. These proteins can have two fates; either be secreted by the bacterium (toxins, exoenzymes, etc.), or be destined for the membranes and the cell wall (intrinsic proteins, etc.);

iv. Support for enzymes and molecule transporters involved in biosynthesis;

v. Respiratory function through electron transport and oxidative phosphorylation in aerobic bacterial species;

vi. Photosynthesis. In photosynthetic bacteria, the pigments and enzymes involved in the photosynthesis process are located in invaginations of the plasma membrane that extend into the cytoplasm. These membranous structures are called chromatophores, or thylakoids.

2.3. Cytoplasm

- The cytoplasm of bacteria lacks certain features observed in the cytoplasm of eukaryotic cells, such as the cytoskeleton, endoplasmic reticulum, and mitochondria. It is a colloidal gel containing all the cytoplasmic constituents and is primarily composed of water (80%) along with organic and mineral substances. The main constituents of the cytoplasm are:

The nuclear region or nucleoid of the cytoplasm contains a long, continuous, circular-shaped filament composed of double-stranded DNA and is called the bacterial chromosome. It serves as the carrier of genetic information. The absence of a nuclear membrane makes the bacterial chromosome highly accessible and facilitates protein synthesis.

Note:

Many bacteria contain other DNA fragments called plasmids. These are small extrachromosomal circular units that are not essential for bacterial growth and metabolism, but they often confer traits or resistance to antibiotics and the production of certain toxins and enzymes.

Bacterial processes of DNA replication, transcription, and translation of messenger RNA into protein sequences are quite similar to what is found in eukaryotes. However, there are specific features related to the organization and structure of prokaryotic genes, as well as the nature of the enzymes involved in these processes, that should be noted.

❖ Ribosomes

Ribosomes are small spherical structures that occupy a significant portion of the cytoplasm, often in the form of chains (polysomes). A ribosome consists exclusively of RNA (60%) and proteins (40%).

Ribosomes are the site of protein biosynthesis. It is at their level that amino acids join together through peptide bonding to form a polypeptide chain.

❖ Cell Inclusions and Storage Granules

Bacteria can accumulate large quantities of organic or mineral substances as energy reserves, which often form granules or inclusions, surrounded by a special single-layer membrane. In general, each bacterial species or group synthesizes a single category of reserve substance.

These granules or inclusions can contain energy-rich organic polymers (e.g., glycogen, poly-β-hydroxybutyrate acid), while other inclusions may contain crystals of inorganic compounds (e.g., polyphosphates, sulfur, iron oxide or magnetite Fe_3O_4), and in some species, there are gas vesicles of the same nature as the gas in the external environment (planktonic bacteria).

2.4. Capsule

The capsule is a coating (a mucous membrane) secreted by certain bacteria that surrounds their walls. It is usually of a polysaccharide nature (polyholosides), but in some cases, it is of a polypeptide nature (D-glutamic acid polypeptide in *Bacillus anthracis*). Its formation is largely influenced by the constituents of the environment. For example, in the absence of carbohydrates, the bacterium does not form a capsule, but once transferred to a carbohydrate-rich environment, it begins to synthesize a capsule.

❖ **Functions**

- Role of protection against phagocytosis and bacteriophages;
- Role of protection against the lethal effects of physical and chemical agents (reduces the effect of desiccation, for example);
- Antigenic role;
- Role in the attachment of bacteria to solid surfaces or other cells.

2.5. Flagella

Bacteria are mobile thanks to specialized locomotor organelles called flagella. These are filamentous appendages composed entirely of proteins. Flagella are long, mobile organelles capable of rotation.

- **Flagellar Insertion Arrangements**

Flagella are more or less long and slender appendages, free at one of their ends and attached to the bacterial cell at the other end. The arrangement of flagella varies among bacteria and influences their mode and speed of movement.

There are four types of arrangements (insertion modes) of bacterial flagella: monotrichous (a single polar flagellum), amphitrichous (one or more flagella at both ends of the cell), lophotrichous (two or more flagella at one end of the cell) (from the Greek "*lophos*" meaning tuft and "*trichos*" meaning hair), and peritrichous (flagella distributed all over the surface of the cell) (where "*peri*" means around).

Bacteria with polar or amphitrichous flagella move rapidly in a straight line or zigzag, while bacteria with peritrichous flagella move slowly in a swirling motion.

- **Structure of flagella**

The flagellum is a helical structure that operates by rotation. It consists of three main parts: the filament, the hook, and the basal body. The filament is the visible part of the flagellum that extends from the surface of the bacterium. It is a long segment with a constant diameter, shaped like a hollow cylinder and composed of flagellin (a globular protein). The filament is attached to a slightly wider hook, made up of a different protein. The third part of the flagellum is the basal body, which anchors the structure in the cell wall and plasma membrane and is responsible for the movement of the filament, thanks to its more complex protein structure (rotor+stator).

- **Functions**

- Mobility in aqueous or gelled environments;
- Chemotaxis. Mobile bacteria possess chemotaxis (chemotaxis or cellular mobility in response to chemical stimuli). Some substances (sugars, amino acids, O2) attract them (positive chemotaxis); others (phenols, acids, bases) repel them (negative chemotaxis). It's worth noting that the stimulus can be light rather than a chemical element, in which case it's called phototaxis.
- Antigenic properties.

2.6. Pili and fimbriae

Many Gram-negative bacteria (and occasionally Gram-positive bacteria) have thin appendages, shorter and straighter than flagella, that serve to attach the bacteria. These structures, composed of a protein called pilin assembled in a helical manner, sometimes around a central core made up of a minor polypeptide called adhesin, come in two types: fimbriae (fringes) (common pili) and pili (hairs) (sexual pili).

The number of fimbriae can vary from a few to several hundred per cell, distributed evenly. Fimbriae allow bacteria to adhere to surfaces to form biofilms or to other cells, including animal cells. Pili (singular: pilus) are generally much longer than fimbriae, and their number does not exceed one or two per cell. Pili connect bacteria during the DNA transfer process from one cell to another, known as conjugation. Apart from their adhesion roles, fimbriae and pili can be major antigenic components in pathogenic bacteria. It is worth noting that type IV pili can be involved in cell motility.

2.7. Endospore (bacterial spore)

Some Gram-positive bacterial genera, such as *Bacillus* and *Clostridium*, can transform into small oval or spherical units that do not divide, remain metabolically inert, and exhibit extreme resistance when the environment becomes depleted of nutrients or when external physicochemical conditions change (elevated temperature, desiccation, lack of water, presence of toxic chemicals, changes in pH, radiation, etc.). This phenomenon is known as sporulation (sporogenesis), and the structures generated are referred to as spores or endospores.

If placed in favorable environmental conditions, these endospores can germinate and give rise to fully active bacterial cells, identical to those that originally produced the endospores. This process is called germination, and the resulting cells are referred to as vegetative cells.

❖ **Morphology and Structure**

The structure of an endospore observed under electron microscopy differs significantly from that of a vegetative cell and consists of two main parts: i. a cytoplasmic (central) region containing primarily the nuclear material along with ribosomes, reserve substances, and enzymes, and ii. the envelopes formed around the sporulation membrane. The arrangement of these envelopes from the inside to the outside is as follows:

- The spore wall, which surrounds the cytoplasmic membrane and contains the normal peptidoglycan that will eventually become the cell's vegetative cell wall after germination.
- The cortex, a thick layer with a monomorphic appearance (10 to 20% of the total); it is composed of a peptidoglycan with a different structure and a high concentration of calcium dipicolinate (promoting spore dehydration, preventing DNA denaturation due to heat and cellular desiccation).
- The coats (internal and external), of a proteinaceous nature, make up about 20 to 25% of the total; their impermeability is responsible for resistance to chemical agents.
- The exosporium, the outermost layer, is a lipoprotein membrane containing 20% sugars (not essential for the spore's survival).

❖ **Sporulation**

Under the influence of environmental stress, cell multiplication ceases (stationary phase), and bacterial sporulation begins. The average duration of sporulation is 7 to 8 hours, and it can be summarized in 7 cytological stages:

- Stages 1 to 3: Sporulation begins with the replication of chromosomal DNA, followed by the condensation of one of its copies at one end of the cell, followed by an asymmetric cell division that forms a double membranous structure (transverse septum) that divides the cell into two unequal parts; one will give rise to the pre-spore and will contain the bacterial chromosome, while the other, corresponding to the vegetative cell carrying the embryonic spore, is called a sporangium.
- Stages 4 to 6: Inside the sporangium, the pre-spore will gradually "mature" by surrounding itself with various envelopes.

- Stage 7: When the endospore is mature, the wall of the vegetative cell ruptures (lyses). The vegetative cell dies and releases the endospore.

The diameter of the endospore can be equal to that of the vegetative cell. It can also be smaller or larger, in the case of a non-deforming spore and a deforming spore, respectively. Depending on the species, the position of the endospore in the vegetative cell can be terminal (formed at one end), subterminal (formed near one end), or central.

❖ **Germination**

Endospores are thermoresistant, able to withstand various chemical agents (antiseptics, antibiotics) and physical factors (UV radiation, high pressure), and their survival duration can be very significant.

The germination of an endospore to become a metabolically active cell involves three stages:

- Activation: Germination is triggered by an agent that destroys the spore coats. This agent can be mechanical (mechanical shock), physical (heat), or chemical (acidity). Often, this activation needs to be accompanied by the presence of nutrients in the environment.
- Initiation: Many components of the spore are degraded by hydrolytic enzymes. After the removal of the cortex, the spore absorbs water, swells, and becomes more permeable.
- Outgrowth: The cell resumes its biosynthesis and doubles its initial volume while shedding the spore coat, with the spore wall becoming the cell wall.

3. Nutrition

Nutrition is a process through which chemical substances called nutrients are acquired from the environment and used in cellular activities such as metabolism (catabolism + anabolism) and cell multiplication (growth). Bacteria, like any other organisms, require sources of these elements. The differences between living organisms concern the primary source of a specific element, its assimilated chemical form, and the quantitative requirements for that element.

Nutrition must be satisfied by two types of substances (nutrients) that a bacterium must find in its environment (natural or synthetic):

i. Elemental substances, which are the building materials of the cell (carbon, nitrogen, minerals, and water) and are used in large quantities; these are macronutrients. They can also be required in smaller quantities or as trace elements, known as micronutrients (trace elements).

ii. Energetic substances that enable the cell to carry out the synthesis of its own components.

3.1. Nutritional needs

3.1.1. Macronutrients

❖ **Carbon**

Carbon is an essential element for all cells. The source of carbon for most bacteria is organic in origin: amino acids, fatty acids, organic acids, sugars, aromatic compounds, etc. These are assimilated by bacteria to synthesize their cellular components. However, a few bacteria are capable of constructing all their cellular structures from carbon dioxide (CO_2). The energy required for this process comes from light or inorganic compounds.

❖ **Nitrogen**

Nitrogen is an element found in proteins, nucleic acids, and several other cellular components. Assimilable nitrogen for bacteria comes in various forms: inorganic, such as ammonia (NH_3) and nitrate (NO_3^-), organic, like the amine groups in organic compounds of the type R-NH_2, or elemental, as some bacteria utilize atmospheric nitrogen (N_2) as their sole nitrogen source.

❖ **Phosphorus and Sulfur**

Phosphorus is a component of phospholipids, nucleic acids, numerous coenzymes, and ATP. It is incorporated into the cell in the form of inorganic phosphate. Sulfur is present in certain amino acids (cysteine, methionine) and, therefore, in proteins in the form of thiol groups (HS^-), in certain vitamins (biotin, thiamine), and coenzymes. Cellular sulfur primarily comes from inorganic sources (sulfate SO_4^{2-}, hydrosulfide HS^-).

3.1.2. Micronutrients

Although required in very small quantities, micronutrients are nonetheless essential for proper cellular function and maintaining the physicochemical balance of the cytoplasm. They generally play a role in catalysis as components of various enzymes. Often metals, they include sodium, potassium, magnesium, chlorine, iron, calcium, manganese, copper, etc. They can be part of an enzyme or coenzyme (iron in respiratory cytochromes, magnesium in chlorophyll) and thus play a significant role in biocatalysis.

3.1.3. Specific needs: growth factors

In addition to the basic elements (micro and macronutrients) mentioned earlier, some bacteria require the presence of organic substances for their growth that they are unable to synthesize, known as growth factors. Based on these requirements, bacteria are classified into two categories: prototrophs, which do not require growth factors, and auxotrophs, which require them.

Growth factors encompass three categories of substances: amino acids, purine and pyrimidine bases (nitrogenous bases involved in the structure of nucleic acids), and vitamins. These growth factors act at infinitesimally small concentrations and have very specific actions.

3.2. Metabolic Diversity of Bacteria

Bacteria are characterized by their significant metabolic diversity, which means they are capable of utilizing a wide range of biochemical reactions to build cellular structures and store energy. Bacteria are grouped metabolically into trophic types based on their nutritional type, energy source, and carbon source.

❖ **According to the energy source**

According to the type of energy used, bacteria are classified into two categories:

- Phototrophs (or photosynthetic), which derive their energy from light;
- Chemotrophs (or chemosynthetic), which use the energy from the oxidation of organic or inorganic chemicals.

❖ **According to the source of carbon**

According to the source of carbon, bacteria are classified into two categories:

- Autotrophs (lithotrophs), which use carbon dioxide (CO2) as their sole source of carbon and use mineral substrates as electron donors (for the reduction of the carbon source into organic compounds);

- Heterotrophic (organotrophs), which use organic carbon substrates.

Based on the sources of energy and carbon, four different nutritional classes are obtained:

i. Photoautotrophs (or photolithotrophs) use light as a source of energy and carbon dioxide as the main source of carbon, their source of electrons is mineral. Example: photosynthetic bacteria (green and purple sulfur or sulfid-reducing bacteria), use sulfur, sulfur compounds, or H_2 to reduce CO_2 and form organic compounds;

ii. Photoheterotrophs (or photoorganotrophs) use light as a source of energy, but are unable to convert CO_2 into sugar; instead, they use organic compounds (alcohols, fatty acids, carboxylic acids, etc.) and carbohydrates as a source of carbon and electrons. Example: green and purple non-sulfur bacteria;

iii. Chemoautotrophs (or chemolithotrophs) use CO_2 as the main source of carbon. They use electrons from reduced inorganic compounds (hydrogen sulfide H_2S for *Thiobacillus thiooxidans*, the nitrite ion NO_2^- for Nitrobacter, or the ferrous ion Fe^{2+} for *Thiobacillus ferrooxidans*, etc.) as a source of energy.

iv. Chemoheterotrophs (or chemoorganotrophs) use specifically as a source of energy the electrons from hydrogen atoms that are part of organic compounds. Almost all bacteria of importance in the food industry, including pathogenic bacteria, are chemoheterotrophs.

4. Growth

Growth can be defined as an orderly increase in all the components of an organism. Bacterial growth results not in an increase in size, as seen in multicellular organisms, but in an increase in the number of cells. Most bacteria reproduce through binary fission (scissiparity): a mother cell divides to give rise to two identical daughter cells. Only a few specific groups of bacteria reproduce differently through budding (budding bacteria), fragmentation (filamentous bacteria), or sporulation (actinomycetes).

4.1. Impact of physical environmental factors on nutrition and bacterial growth

The activity of bacteria and therefore their growth varies depending on physical factors (temperature, pH, osmotic pressure, radiation, etc.) and chemical factors (especially nutrient availability) in the environment. These factors can either prevent, inhibit, or promote bacterial growth.

4.1.1. Temperature

Temperature greatly influences cell division and cellular metabolism. Each bacterium has a minimum temperature below which there is no growth, an optimal temperature at which growth is fastest, and a maximum temperature[2] above which growth is not possible. These three temperatures are called cardinal temperatures and are characteristic of each bacterium. Based on the optimal growth temperature, bacteria are generally categorized into three groups[3] :

- **Psychrophiles** : among the bacteria that can thrive at 0°C, we can distinguish:
  - Strict psychrophiles: can grow at temperatures between -10°C and 20°C, but their optimal growth temperature is within the range of 10 to 15°C.

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[2] In general, the optimal temperature is closer to the maximum temperature than the minimum temperature.
[3] This classification has no strict boundaries. There can be overlaps between the groups.

- Psychrotrophs: can grow at temperatures ranging from 0 to 35°C, with their optimal growth temperature falling between 20 and 30°C.

❖ **Mesophiles** : they can thrive at temperatures ranging from 10 to 45°C, encompassing the majority of bacteria, including pathogens. They have adapted to life within the animal body and consequently have optimal temperatures close to those of their host.

❖ **Thermophiles** : are bacteria capable of growing at high temperatures and are unable to grow at temperatures lower than 45°C[4].

4.1.2. pH

The action of pH occurs at three levels: the environment, membrane permeability, and metabolic activity. Indeed, the availability of certain nutrients (especially metal ions) can be altered by ionic balance, ATP synthesis is closely dependent on ion pumps (H^+), and enzymatic activity is highly sensitive to pH variations.

Bacteria mostly multiply in a neutral pH range (6.5-7.5) (neutrophiles). These limits can be broader. Other bacteria have preferences for alkaline (alkaliphiles or basophiles) or acidic (acidophiles) pH levels.

4.1.3. Oxygene

The gas requirements of bacteria are most precise when it comes to oxygen: some bacteria absolutely need oxygen; they are strict aerobes. Others can only thrive in the absence of oxygen, and they are strict anaerobes. Some are facultative aerobes (or aero-anaerobes); they can multiply in the presence or absence of oxygen. Finally, other bacteria known as microaerophiles only reproduce in the presence of a low concentration of oxygen.

4.1.4. Moisture (water activity, Aw)

Water is used by bacteria in two ways: as a solvent for nutrients, thereby enabling their transport and availability, and as a chemical agent in hydrolysis reactions.

The amount of available water can be measured. Water activity (Aw) is used as a quantitative parameter for available water[5]. Bacteria require a certain level of humidity; in the presence of a low water activity (Aw), they respond by slowing down their growth. In general, bacteria require an Aw equal to or greater than 0.95.

4.1.5. Pressure

❖ **Osmotic Pressure**

The concentration of solute molecules in the environment surrounding the bacterium is an essential growth factor. The bacterial cytoplasm is rich in molecules of all kinds, and as a result, the osmotic pressure there is generally higher than in the environments where bacteria live. The salinity range between 0 and 3% is the one in which the vast majority of bacteria can grow.

Other physical factors, such as mechanical pressure and radiation, can also influence bacterial growth and development.

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[4] Thermotolerant organisms are all mesophilic organisms capable of growing at temperatures > 45°C.

[5] A_w is defined as the ratio of the saturated vapor pressure of the medium to the saturated vapor pressure of pure water at the same temperature ($A_w = P/P_0$). This ratio is less than or equal to 1.

4.2. Bacterial growth kinetics

4.2.1. Bacterial kinetics parameters

In order to quantify bacterial growth, the number of cells (or biomass denoted as X), or alternatively, the changes in their metabolites (substrates, products, cellular constituents) are determined. Monitoring the variation in biomass quantity over time (t) allows for the construction of a growth curve, X = f(t), referred to as a growth curve.

- **Exponential growth**

If an initial biomass quantity X_0 is cultured at time t_0 under stable physicochemical conditions where nutrients are constantly replenished and waste is continually removed, the number of initial cells will increase following a geometric progression according to the equation:

$$X_n = X_0 2^n \quad (1)$$

With X_n : The number of cells after n divisions is equal to n, where n is the number of generations (divisions)
The logarithmic expression of this equation is often used to plot the growth curve:

$$\log X_n = \log X_0 + n \log 2 \quad (2)$$

So the number of divisions. $n = (\log X_n - \log X_0)/\log 2 \quad (3)$

This type of growth is called exponential growth. And from this equation, we can define the following parameters:

The generation time, denoted as G[6]: $G = (t_n - t_0)/n \quad (4)$

This is the time required for a bacterium to undergo division, and therefore for the occurrence of a generation. This time varies depending on the species and the physicochemical factors of the environment.
The specific growth rate, denoted as μ:

$$\mu = \log(X_n/X_0)/t \quad (5)$$

This is the growth rate reported in terms of biomass and volume unit ($g.L^{-1}.h^{-1}$ or number of $cells.h^{-1}$). μ_X is a factor dependent on the bacterial species and culture conditions.

4.2.2. Growth curve of bacteria in a non-renewable environment

The growth of bacteria is limited. After a certain period of culture, which varies depending on the species, it stops. This is because we work with a limited volume of culture medium[7] with precise and limited quantities of nutrients. The medium eventually becomes depleted. This is what is known as growth in a non-renewable medium.

On a non-renewed culture medium, the cell concentration over time follows a typical growth curve, which generally includes four phases: a lag phase, an exponential phase, a stationary phase, and a decline phase, as depicted in Figure 1.

- **Latency Phase:** Initially, the number of bacterial cells varies very little because they do not begin reproducing immediately after being introduced into a new environment[8]. This period during which cells do not divide, or divide very little, is called the latency phase, and its duration is relative. This latency time

[6] Also denoted as θ, it is expressed per unit of time (e.g., hours).
[7] Solution of various nutrients promoting cell growth.
[8] It depends on the cultivated species and the growing conditions.

corresponds to a high metabolic activity of the cells, as they establish the enzymes that will be involved in cellular activities (biosynthesis, cell division).

- **Exponential phase:** After the lag phase, cells begin to divide and enter a period of growth called the exponential phase. This is the period during which cell reproduction is most intense, and the generation time G is constant and has the shortest duration, and μ is maximal. It is during this phase that bacterial metabolic activity is most intense.
- **Stationary phase:** Growth eventually slows until the number of new cells and the population stabilize. At this point, cell metabolic activity also decreases. This period of equilibrium is called the stationary phase. This phase can be explained by the depletion of nutrients, the accumulation of waste products, and unfavorable changes in the conditions of the medium (pH in particular).
- **Decline phase:** If the culture continues after the stationary phase, it reaches a stage where the number of bacteria that die exceeds the number of new bacteria; the population then enters the decline or decay phase. This phase continues until X decreases significantly, or until all cells die.

The time that these four phases last varies depending on the bacterial species. Thus, in some species, they can occur in just a few hours or days, while in other species they can occur over even longer periods.

In a synthetic medium containing a mixture of two carbon substrates, an abnormal diphasic curve can be observed, as if two growths followed each other, this phenomenon is called diauxie.

In a bacterial population, cells do not divide at the same time or at the same rate. This lag makes growth asynchronous. Synchronous growth can be induced experimentally under certain conditions.

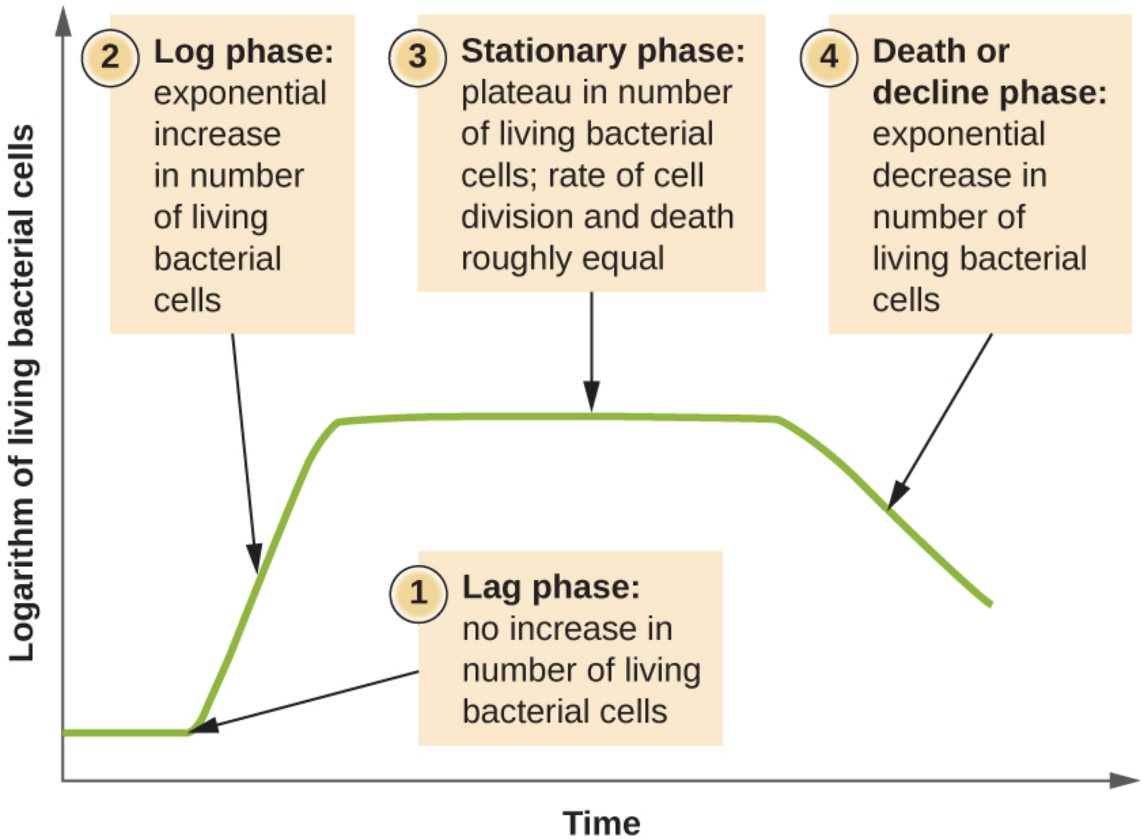

Figure 1. Growth curve of a bacterial population as a function of time (logarithmic scale), on a non-renewed culture medium.