1- Metabolism concept

The maintenance of cellular integrity, growth and reproduction of living organisms, involve the synthesis of cellular material, which depends on nutrients entering the cell and undergoing a series of chemical modifications. The sum of these modifications constitutes metabolism.

A cell's metabolism encompasses the degradation (catabolism) and elaboration (anabolism) of macromolecules.

Catabolism is a series of chemical reactions that result in the degradation of organic or inorganic compounds absorbed by organisms into small molecules that release energy. In contrast, anabolism corresponds to assimilation or biosynthesis, requiring sufficient energy to synthesize complex molecules from simple metabolites derived from degradation.

Biosynthesis takes place either autotrophy, from inorganic elements such as CO_2, or heterotrophy, from reduced organic molecules. In the case of phototrophic or photosynthetic bacteria, energy comes from light.

Microorganisms are either autotrophic or heterotrophic. However, some mixotrophic prokaryotes can use autotrophic or heterotrophic carbon sources, depending on environmental conditions.

For some microorganisms, a single carbon source covers all the carbon requirements for biosynthesis; these are known as prototrophs. Others require, in addition to the main carbon source, organic compounds they cannot synthesize, such as amino acids, vitamins, etc. They are called auxotrophs (see nutrition section).

All biochemical reactions are catalyzed by enzymes, which reduce activation energy and accelerate reaction speed.

Enzymes are made up of a specific protein part (in some cases RNA) called an apoenzyme and a mineral compound (metal ion: iron, manganese, magnesium, copper, etc.) or organic compound (NAD, NADP, FAD, etc.) called a cofactor.

The apoenzyme is permanently linked to the cofactor by covalent bonds (e.g. the heme of cytochromes); this is the **prosthetic group**. When the cofactor is weakly attached to the apoenzyme, it can detach from the enzyme after product formation and transport one of the products to an enzyme.

The most common coenzyme is nicotinamide adenine dinucleotide (NAD), which is easily dissociated from the enzyme and plays a role in electron transfer (Table 1).

Table 1: Principal coenzymes

Coenzyme name	Abréviations	Vitamines	Groupements transférés
Nicotinamide adenine dinucleotide	NAD	Vitamine B3 (nicotinamide or niacinamide)	H
Nicotinamide adenine dinucleotide phosphate	NADP	Vitamin B3 (nicotinamide or niacinamide)	H
Flavin mononucleotide	FMN	Riboflavin	H
Flavin adenine dinucleotide	FAD	Riboflavin	H
Adenine triphosphate	ATP	Lipoic acid	P et AMP
Coenzyme A Thiamine pyrophosphate	CoA TPP	Panthoteic acid Thiamine	Acyl and acetate Carbon 1 grouping

2-Energy metabolism

2-1- General principles

Metabolic energy production involves redox reactions between electron donors and acceptors during respiration, fermentation or photosynthesis. This energy is usually stored in the form of energy-rich phosphate bonds, mainly in the form of adenosine triphosphate (ATP).

ATP synthesis is an endergonic reaction (ΔGp' : + 44 kj.mol-1) which requires energy from catabolism:

ADP + Pi ⟶ ATP + H_2O

Whereas the energy released during hydrolysis of ATP to ADP is ΔGp0' is - 32 kj/mol. Other energy-rich molecules can also be used, such as phosphoenolpyruvate (PEP), acetyl phosphate and acetyl CoA.

Three trophic groups are associated with this energy production. If the energy source is a reduced organic molecule whose oxidation releases electrons to be given to an acceptor (organic or inorganic), we speak of a chemoorganotroph. The latter trophic group produces energy by respiration (aerobic or anaerobic), or by fermentation. If the energy source is a reduced inorganic substrate, the oxidation of which provides electrons to be given to a terminal acceptor, we speak of chemolithotrophy.

Chemolithotrophs produce energy through aerobic or anaerobic respiration. Energy can also come from light, a process known as phototrophy, in which a flow of electrons is generated by the absorption of photons by photoreceptors.

Generally, the same reduced organic or inorganic molecule is used as a source of energy and electrons.

We can define 5 trophic groups according to the sources of energy, electrons and carbon used: chemoorganotrophs (chemoorganoheterotrophs), chemolithotrophs (chemolithoheterotrophs), photoorganotrophs (photoorganoheterotrophs), chemolithotrophs (chemolithoautotrophs), photolithotrophs (photolithoautotrophs) (see previous lessons).

2-2- Penetration of substances

In the bacterial cell, the respiratory chain enzymes are located at the cytoplasmic membrane (dehydrogenases, cytochromes, flavoprotein, cytochrome oxidase). The others are located in the cytoplasm.

Food can only be assimilated by bacteria if it penetrates inside the cell. Their high molecular weight means they must be broken down by hydrolytic enzymes excreted by the bacteria into the external environment.

a) enzymes active on proteins (proteinases) and peptides (peptidases);

b) glucidases active on holosides and heterosides (glucosidases), starch (amylase), cellulose (cellulase), pectins (pectinases), glycogen (glycogenase), complex polysaccharides (hyaluronidase on hyaluronic acid);

c) nucleases active on nucleic acids;

d) amidases active on urea (urease), hippuric acid (hippuricase);

e) esterases active on triglycerides (lipases) or lecithins (lecithinases or phospholipases).

Nutrients must cross the peptidoglycan (by diffusion or by receptors) and the cytoplasmic membrane in Gram-positive bacteria; the outer membrane (via porins), the peptidoglycan (by diffusion or receptors) and the plasma membrane in Gram-negative bacteria.

To achieve this, bacteria have developed active transport systems, as passive diffusion would be too slow to meet the needs of bacterial metabolism. These means of active transport are:

a) membrane proteins acting as specific receptors for the various substrates ;

b) enzymes providing the energy required for transmembrane transfer. The latter system is known as permease.

2-3- Respiration in microorganisms

Metabolism is more diversified in prokaryotic microorganisms than in eukaryotic microorganisms:

- the energy source (electron donors) is necessarily organic for eukaryotic microorganisms, whereas in prokaryotes it can be organic or inorganic (chemoorganotrophs or chemolithotrophs).
- the terminal electron acceptor is generally oxygen (O_2) in eukaryotes, whereas in prokaryotes there is a diversity of terminal electron acceptors in addition to O_2, such as nitrate, sulfate, iron..., thus defining the diversity of anaerobic respirations.
- the composition of respiratory chains is highly diversified in prokaryotes, whereas it is constant in eukaryotes.

2-3-1- Aerobic respiration in chemoorganotrophic microorganisms

In chemoorganotrophic microorganisms, electrons originate from the oxidation of organic molecules and are transferred to O_2 by a chain of intermediate transporters (respiratory chain). This property is widespread in the living world. During this transfer, energy is conserved in the form of ATP, produced by oxidative phosphorylation.

2-3-1-1- Carbohydrate oxidation

Most carbohydrates can be converted to glucose by chemoorganotrophic microorganisms. These microorganisms use several metabolic pathways to break down glucose and other sugars, the most important of which are: the Embden-Meyerhof pathway known as glycolysis, the pentose phosphate pathway (hexose monophosphate or Warburg-Dickens-Horecker pathway) and the Entner-Doudoroff pathway (2-keto-3-desoxy-6-phosphogluconate, CDPG).
These three pathways enable prokaryotes to convert sugars into pyruvate and intermediate metabolites, either aerobically or anaerobically. However, they differ in the number of ATPs and the quantity and type of reduced cofactors produced.
The choice of which of these pathways to use depends on the microorganism's enzymatic baggage.

- **Embden-Meyerhof (or glycolysis or Embden-Meyerhof-Parnas) pathway**

Found in most living organisms. Located in the cytoplasm, the glycolysis pathway leads to the conversion of a six-carbon sugar (glucose or other hexoses) into 2 molecules of pyruvate, with the formation of ATP by phosphorylation of the substrate and reduced coenzymes (Figure 1).
During this pathway, there are:
- Hexose activation: glucose is activated to glucose-6-phosphate, then to fructose-6-diphosphate via fructose 6-phosphate hexokinase.

- Hexose cleavage: fructose 1-6- diphosphate is cleaved by an aldolase into 2 compounds, dihydroxy-acetone phosphate and 3-phosphoglyceraldehyde. The isomerization reaction is carried out by a triose phosphate isomerase, which shifts the equilibrium towards 3-phosphoglyceraldehyde (or glyceraldehyde 3 phosphate, 3PGA).
- Oxidation of triose phosphate: catalyzed by a dehydrogenase in the presence of phosphate and NAD^+. Followed by phosphorylation, and NAD^+ is reduced to NADH, H^+.
- Formation of pyruvate and 2 ATP molecules. This production of ATP in the cytoplasm, coupled with the breakdown of an energy-rich substrate molecule, is known as substrate phosphorylation.

The catabolism of glucose to pyruvate can be represented by the simple equation:

Glucose + 2ADP + 2Pi + 2 NAD^+ ⟶ 2 pyruvate + 2 ATP + 2 (NADH, H^+)

- **Pentose phosphate pathway (or hexose monophosphate or Warburg-Dickens-Horecker pathway)**

The pentose phosphate or hexose monophosphate pathway is used by some bacteria in conjunction with glycolysis. It is used for energy production and biosynthesis reactions (anabolism).

It begins with the oxidation of glucose 6-phosphate to 6-phosphogluconate and continues with the oxidation of the latter product to a pentose (ribulose 5-phosphate), and CO_2 (Figure 2). The ribulose 5-phosphate is then converted into a mixture of 3- and 7-carbon phosphate sugars (3C and 7C). 2 key enzymes are involved in this pathway: transketolase, which catalyzes the transfer of 2-carbon units from a ketose, and transaldolase, which transfers a 3-carbon group from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate.

3 glucoses 6 phosphate are converted into 2 fructoses 6 phosphate, one glyceraldehyde 3 phosphate and 3 CO_2 molecules, as shown in the following equation:

3 glucoses 6P + 6 $NADP^+$ + 3 H_2O ⟶ 2 fructoses 6P + Glycéraldéhyde 3P + 3CO_2 + 6(NADPH, H^+).

Fructose 6 phosphate is converted back into glucose 6 phosphate and glyceraldehyde 3 phosphate into pyruvate by the enzymes of glycolysis.

Glyceraldehyde 3 phosphate can return to the pentose phosphate pathway to form glucose 6 phosphate.

For the ATP balance, 3 molecules of ATP are consumed to phosphorylate 3 molecules of glucose (**3 glucose + 3 ATP ⟶ 3 glucose 6P + 3 ADP**) but the production of only 2 ATP molecules to convert 3-glyceraldehyde into pyruvate via glycolysis.

This pathway is important for the bacterium and its role is to:

- supply NADPH, H+ as a source of electrons for the reduction of molecules during biosynthesis;
- supply 4- and 5-carbon sugars used in the biosynthesis of compounds such as amino acids and vitamin B6. Ribose-5-phosphate essential for nucleic acid synthesis.

Note: Glucose 6-phosphate comes from glucose phosphorylation (consumption of one ATP/glucose molecule).

- **Entner-Doudoroff pathway**

This pathway is the least represented in prokaryotes. It is involved in alcoholic and heterolactic fermentation.

Glucose 6-phosphate is formed by phosphorylation of glucose, which is then converted into 6-phosphogluconate (Figure 3). The latter product is then dehydrated to form 2-keto-deoxy-6-phosphogluconate. The latter is cleaved by a keto-deoxy-phosphogluconate aldolase into pyruvate and glyceraldehyde 3-phosphate (3 PGA); 3 PGA is converted into pyruvate.

The energy balance is therefore 1 ATP, 1 NADPH, H+ and 1 NADH, H+ per molecule of glucose degraded into 2 molecules of pyruvate.

- Catabolism of disaccharides

Disaccharides are broken down by specific enzymes, e.g. β-galactosidase for lactose, maltase for maltose and invertase for sucrose. Fructose from sucrose is converted by phosphorylation into fructose phosphate, which joins glycolysis. Galactose from lactose is epimerized into glucose.

- Polysaccharide catabolism

Microorganisms have specialized in the degradation of polysaccharides such as starch and cellulose.

- Starch is degraded by amylase; an extracellular enzyme that can be an α-amylase (splits the molecule at any point (endoamylase) at α- (1-4) bonds), a β-amylase (cuts from its end; exoamylase) or a γ-amylase (attacks α- (1-4) or α- (1-6) bonds).

- Tricarboxylic acid cycle (Krebs cycle)

The Krebs cycle exists in higher organisms and some microorganisms (Figure 4). A great deal of energy is released when pyruvate is degraded to CO_2 in the presence of O_2.

In prokaryotic microorganisms, glycolysis and the Krebs cycle take place in the cytoplasm, whereas in eukaryotes, glycolysis takes place in the cytoplasm and the Krebs cycle in the matrix of the mitochondrion, an organelle absent in bacteria.

Pyruvate is first oxidized by pyruvate dehydrogenase to form CO_2 and acetyl CoA, an energy-rich molecule made up of CoA and acetic acid. Acetyl CoA is produced by the breakdown of lipids, carbohydrates and amino acids.

- Citrate formation: acetyl CoA is condensed to oxaloacetate (derived from pyruvate by carboxylation) by a lyase.

- Formation of α-ketoglutarate: citrate is converted to cis-aconitate and then to isocitrate by aconitase. The latter undergoes decarboxylation to α-ketoglutarate by oxalosuccinate decarboxylase.

- Formation of succinate from α-ketoglutarate, which is first converted to succinyl CoA by decarboxylation with ketoglutarate dehydrogenase, then to succinate by deacylation with succinate thiokinase.

- Formation of oxaloacetate through a series of redox reactions. Succinate is oxidized to fumarate by succinate dehydrogenase, then hydrated to malate by fumarase. Malate is again oxidized to oxaloacetate by malate dehydrogenase.

Results: for one cycle, one molecule of acetyl-CoA yields 2 molecules of CO_2 and 8 H^+ in the form of 2 molecules of NADH, H^+, 1 NADPH, H^+ and 1 $FADH_2$.

Total oxidation of a glucose molecule via the glycolysis pathway and the Krebs cycle produces 6 CO_2 molecules, 4 ATP, 8 NADH,H^+, 2 NADPH,H^+ and 2 $FADH_2$.

2-3-1-2- Lipid oxidation

Lipids can be broken down by lipases into fatty acids and glycerol, which are present in certain bacteria such as *Clostridium* and *Bacillus*, and in some yeasts and molds.

Glycerol joins glycolysis and fatty acids are oxidized by β-oxidation (Figure 5). The fatty acid is esterified to acyl-CoA by coenzyme A. Acyl-CoA is then oxidized to the β-position in 3 steps (2 dehydrogenations and 1 hydration). Further esterification releases an acetyl-CoA and an acyl-CoA that has lost 2 carbon atoms. A new β-oxidation cycle thus begins.

Acetyl-CoA is metabolized by the Krebs cycle.

2-3-1-3- Protein oxidation

Proteins are degraded by extracellular proteases (or extracellular proteinases) and peptidases to produce oligopeptides and, above all, amino acids. Exopeptidases hydrolyze proteins from the C-terminal or N-terminal end, while endopeptidases cut bonds within the peptide chain. The amino acids and oligopeptides resulting from this action are absorbed. The latter are hydrolyzed into amino acids by intracellular peptidases.

Amino acids are either incorporated into bacterial protein biosynthesis, or metabolized via the 2 main pathways:

- Oxidative deamination: leads to the production of an organic acid (intermediate metabolites) and ammonia (NH_3) (Table 2). Intermediate metabolites are then oxidized via the Krebs cycle.
- Decarboxylation: the second major route of amino acid degradation. The resulting metabolites are often volatile amines responsible for foul odors and putrefaction.

Table 2: Organic acids from amino acid deamination

Amino acids	Intermediate metabolites
Alanine, glycine, cysteine, sérine, thréonine	Pyruvate
Asparagine, aspartate	Oxaloacetate
Tyrosine, phenylalanine, aspartate	Fumarate
Isoleucine, methionine, threonine, valine	Succinate
Glutamate, glutamine, histidine, proline, arginine	α-ketoglutarate
Isoleucine, leucine, tryptophane, lysine, phenylalanine, tyrosine	Acetyl-CoA

2-3-1-4- Energy synthesis

The actual balance is the equivalent of only 4 molecules of ATP from the oxidation of glucose into 6 moles of CO_2. Most of the ATP produced comes from the oxidation of NADH, H^+ and $FADH_2$ in the electron (e^-) transfer chain.

In bacteria, the e- transfer chain involved in respiration is part of the cytoplasmic membrane (Figure 6).

These include cytochromes (iron-containing proteins that accept and transfer e- by alternating reduction and oxidation of the iron atom), iron-sulfur proteins such as ferredoxins and quinones (aromatic compounds that can undergo reversible reactions). These are specialized molecules that channel e-. This flow of e- provides energy as they move from a higher to a lower energy level.

When NADH, H^+ is oxidized by NAD dehydrogenase, its two hydrogen atoms ($2H^+ + 2\ e^-$) are captured by the FAD (or FMN) of flavoproteins, which decouple them: the protons (H^+) are expelled into the periplasm and the $2e^-$ are transferred to Fe-S proteins. 2 protons are then

extracted from the cytoplasm by the dissociation of intracellular water and captured by coenzyme Q, along with e^- from Fe-S proteins. The 2 hydrogen atoms of coenzyme Q are again dissociated into $2H^+$ and $2e^-$, the H^+ are released into the periplasm and the e^- are captured by cytochrome b556. This is a 2-phase process, as the cytochrome can only transport one e^- at a time. The cytoplasm b556 electrons are transferred to cytochrome oxidase (Cytochrome O), a dehydrogenase that completes the transport of e^- into the respiratory chain: O_2 is then reduced to H_2O ($½\ O_2 + 2\ H^+ + 2\ e^-$).

The proton gradient created is favored by the arrangement of e- and hydrogen transporters in the respiratory chain. The result is an accumulation of opposite electrical charges on either side of the cytoplasmic membrane: H^+ ions on the outer side, responsible for an acidic pH and a positive electrical charge, while HO^- ions are concentrated on the inner side, with a basic pH and a negative electrical charge.

This difference in charge generates a pH gradient and an electrochemical potential, known as the driving proton force. This driving proton force can be used for ATP synthesis or directly to perform work, such as nutrient transport and cell movement.

ATP synthesis is achieved by the return of protons (H^+) via proton pores associated with transmembrane enzyme complexes known as ATP synthetases. This process is known as oxidative phosphorylation.

2-3-2- Aerobic respiration in chemolithotrophic microorganisms

Chemolithotrophic microorganisms are all prokaryotes (*Bacteria* and *Archaea*), most of which are chemoautotrophs. They use the oxidation of reduced mineral compounds to produce energy, transferring electrons to O_2 via a membrane respiratory chain as in the case of chemoorganotrophic microorganisms.

The main reduced mineral compounds used are :

- reduced nitrogen compounds, used by nitrifying bacteria (nitriting and nitrating bacteria);
- reduced sulfur compounds such as sulfide (HS^-, S^{2-}, H_2S) and thiosulfate ($S_2O_3^{2-}$) are used by aerobic sulfo-oxidizing bacteria. This bacterial group includes both autotrophs and heterotrophs;
- reduced iron or ferrous iron (Fe^{2+}), used by autotrophic aerobic ferro-oxidizing bacteria as an electron donor, thus oxidized to ferric iron (Fe^{3+}) via a respiratory chain that diverts electrons to O_2.

Energy production in chemolithotrophic microorganisms takes place by transferring electrons from oxidation of the reduced mineral compound to O_2 via a respiratory chain located in the

cytoplasmic membrane. The energy produced remains low compared with that produced by chemoorganotrophs.

2-3-3- Anaerobic respiration

In anoxic environments (absence of O_2) in the absence of light, microorganisms can use anaerobic respiration or fermentation to produce energy.

During anaerobic respiration, electrons from the oxidation of the electron donor are transferred via a respiratory chain to oxidized mineral acceptors (nitrate, sulfate, ferric iron, CO_2...) and sometimes organic compounds (fumarate...).

Examples of anaerobic respiration: iron respiration, nitrate respiration NO_3^- ...

Dissimilatory nitrate reduction

The use of nitrate as a terminal electron acceptor is widespread in prokaryotes. The first step, leading to nitrite (NO_2^-), generates energy via the respiratory chain. The nitrites formed can follow 2 routes: (i) they can accumulate in the external environment or be reduced to NH_3 (ii) they can be reduced to N_2 via the membrane respiratory chain and released into the atmosphere. In fact, bacteria performing anaerobic respiration have e^- transport systems containing cytochromes, quinones, Fe-S proteins and others.

$NO_3^- + 2\ e^- + 2H^+ \longrightarrow NO_2^- + H_2O$

$NO_2^- + 3\ NADPH, H^+ + H^+ \longrightarrow NH_3 + 2\ H_2O + 3\ NADP^+$

$NO_2^- + e^- + 2H^+ \longrightarrow NO + H_2O$

$NO + e^- + H^+ \longrightarrow N_2O + ½\ H_2O$

$N_2O + e^- + H^+ \longrightarrow ½\ N_2 + ½\ H_2O$ (N_2: gaz)

The energy content of nitrate respiration is very high, close to that of aerobic respiration. The two types of respiration are in competition. In the presence of O_2, aerobic respiration is favored until the latter is exhausted. Other bacteria perform obligatory anaerobic respiration (unable to use O_2).

Note: released N_2O can be converted back to NO by sunlight, which reacts with ozone to give NO_2^- which returns to earth in the form of acid rain (HNO_2).

2-4- Fermentations

Fermentation is a form of energy metabolism, usually anaerobic, requiring an organic electron donor. These electrons are transported to an intermediate oxidized organic molecule resulting from the incomplete degradation of the initial substrate (no exogenous electron acceptors).

Fermentations produce large quantities of gases and volatile products such as CO_2, H_2, alcohols, organic acids, etc. (Figure 7), which are released into the external environment. The low energy yield that characterizes fermentations forces fermenting microorganisms to degrade large quantities of organic matter.

Energy conservation mechanisms

ATP is synthesized by phosphorylation at the substrate level. For the same substrate, fermentation is less efficient than aerobic respiration. For example, for one mole of glucose, brewer's yeast releases 2872 kJ by breathing, but only 236 kJ by fermentation.

Diversity of fermentations

Fermentations are classified according to the products formed or substrates consumed. Fermentation processes are highly diversified (Table 3), and widespread among microorganisms.

During fermentation, sugars are generally oxidized to pyruvate. This metabolite or its degradation products become electron acceptors for the reoxidation of reduced coenzymes (NADPH, H^+, NADH, H^+).

Some microorganisms are facultative fermentative (capable of aerobic or anaerobic respiration), while others are obligatory fermentative (strict anaerobic and aerotolerants).

Table 3: Fermentation examples

fermented substrate	Fermentation denomination	Fermentation products	Examples of microorganisms
Sucres			
	Alcoholic	Ethanol, CO_2	Yeast, *Zymomonas*
	Lactic	Lactic acid and sometimes ethanol, acetic acid, CO_2	*Lactobacillus, Lactococcus, Leuconostoc*
	Acetic	Acetic acid	*Clostridium thermoaceticum*
	Mixtes acid	formic acid, acetic acid, lactic acid, succinic acid, ethanol, CO_2, H_2	*Escherichia, Salmonella, Shigella, Proteus*
	2,3, butanediol	2,3, butanediol, lactic acid, formic acid, éehanol, CO_2, H_2	*Enterobacter, Serratia, Erwinia*
Acides organiques			
Lactic acid	Propionic	Acide propionique, CO_2	*Clostridium propionicum*
Malic acid	Malo-lactic	Lactic acid, CO_2	*Leuconostoc oenos*
Acides aminés			
Alanine		Propionic acid, acetic acid, NH_3, CO_2	*Clostridium propionicum*
Arginine		Ornithine, CO_2, NH_3	*Clostridium, Streptococcus*

2-5- Photosynthesis

Phototrophic microorganisms use solar energy as a source of energy, which they capture and convert into chemical energy. Most phototrophs are photo-autotrophs.

Photosynthesis takes place in 2 phases: a light phase during which light energy is conserved in the form of ATP and reduced coenzymes (NADPH, H^+), and a dark phase during which CO_2 is reduced, organic compounds are synthesized, and ATP and reduced coenzymes are consumed.

The presence or absence of O_2 release divides phototrophic microorganisms into oxygenic and anoxygenic phototrophs.

1- In oxygenic phototrophs, such as Cyanobacteria and photosynthetic unicellular eukaryotes, the electron donor for coenzyme reduction is water, and photosynthesis is accompanied by O_2 release. In this group, reaction centers containing chlorophyll a and electron transporter chains are located in thylakoids in the cytoplasm.

2- In anoxygenic phototrophs, which are all prokaryotes in the bacteria family, electron donors can be low-molecular-weight organic compounds that also serve as a carbon source. These are photo-organotrophic or photo-heterotrophic bacteria. Another category of bacteria uses inorganic compounds such as H_2, reduced sulfur compounds (anoxygenic photolithotrophs). The reaction centers contain bacteriochlorophyll, which is associated with chains of electron transporters located at the cytoplasmic membrane.

3- Biosynthesis (anabolism)

A significant proportion of the energy produced during catabolism is used to synthesize the cell's constituents. Autotrophic microorganisms synthesize carbohydrates, proteins, lipids, etc. from inorganic or mineral molecules (CO_2, ammonium, sulfate, etc.). Whereas heterotrophic microorganisms depend on organic molecules produced by autotrophs. The macromolecules that make up their various structures are assembled from their subunits: amino acids for proteins, nucleotides for nucleic acids, sugars for polysaccharides, and glycerol and fatty acids for lipids.

Subunits can be obtained from the external environment, but many bacteria can also synthesize them. These syntheses and the constitution of macromolecules require specific enzymes and energy.

The anabolic chains used by bacteria are quite numerous, but not all are required at the same time, depending on the composition of the environment. Positive and negative regulation systems for the genes encoding all these enzymes therefore exist (regulons), and are

influenced by the presence or absence of certain substrates (polymers, simple elements, ions) in the external environment.

3-1- Autotrophic microorganisms: CO_2 assimilation

This category of microorganisms can assimilate CO_2 in several ways:

(i) the ribulose 1,5-diphosphate or Calvin cycle ;

(ii) the inverse tricarboxylic acid cycle;

(iii) the acetyl CoA reductive pathway;

(iv) the 3-hydroxypropionate cycle;

(v) the C4 pathway.

3-1-1- The ribulose 1,5-diphosphate pathway (Calvin or Calvin-Benson cycle)

This pathway enables autotrophic microorganisms (cyanobacteria, anoxygenic phototrophic bacteria, chemolithotrophs) and plants to fix CO_2 (Figure 8).

The key enzyme in this cycle is ribulose 1,5-biphosphate carboxylase (RuBisCo). It catalyzes the carboxylation of ribulose 1,5-diphosphate. The cycle comprises 3 phases:

Phase 1: fixation of 3 molecules of CO_2 with 3 molecules of ribulose 1.5 di-phosphate, to form 6 molecules of 3-phosphoglycerate.

Phase 2: reduction phase, the 6 molecules of 3-phosphoglycerate are reduced to 3-phosphoglyceraldehyde (PGA), 1 molecule is reserved for biosynthesis and the other 5 for regenerating the 3 ribulose 1.5 di-phosphate molecules.

Phase 3: 1 molecule of C3 (PGA) is synthesized from 3 molecules of CO_2.

$3CO_2 + 9ATP + 6\ NADH, H^+ (or\ 6NADPH,H^+) \longrightarrow 1PGA + 9ADP + 8Pi + 6NAD^+ (or\ 6NADP^+)$

Note: in prokaryotes and phototrophs, the Calvin cycle takes place in the cytoplasm, whereas in phototrophic eukaryotes, it takes place in the chloroplast stroma.

3-1-2- Inverse tricarboxylic acid cycle

This cycle exists in some prokaryotes, such as sulfate-reducing bacteria and archaea, green phototrophic bacteria ..... These organisms fix CO_2 during the reverse Krebs cycle.

3-2- Heterotrophic microorganisms

This category of microorganisms uses organic compounds for growth. These compounds provide energy (ATP, reduced coenzymes, proton-motive force) and carbon skeletons for biosynthesis.

Most heterotrophic microorganisms must excrete extracellular enzymes or exoenzymes, which break down macromolecules into smaller, more easily transportable molecules (monomers) (see section 2-2).

Simple molecules (hexoses, amino acids, acetyl-CoA, glycerol, ...) resulting from metabolic activities become precursors for biosynthesis. They feed the central metabolic pathways to produce the monomers needed to synthesize macromolecules.

3-2-1-Central metabolic pathways and carbon skeleton formation

Precursor or intermediate metabolites are all synthesized by a series of reactions referred to as central metabolites. Four pathways (Figure 9) are common to the majority of eukaryotic and prokaryotic microorganisms:

- glycolysis ;
- gluconeogenesis ;
- the tricarboxylic acid cycle (Krebs cycle) ;
- the pentose phosphate pathway.

In *E. coli*, glycolysis provides 6 precursors, the Krebs cycle: 3 precursors and the pentose phosphate cycle: 2 precursors and acetyl CoA; [glucose 6P, fructose 6P, ribose 5P, erythrose 4P, triose P, 3 phosphoglycerate, 7 phospho enolpyruvate, pyruvate, acetyl CoA, α-ketogutarate, succinyl CoA, oxaloacetate].

3-2-3- Amino acid biosynthesis

If amino acids are not present in the environment or cannot be assimilated by bacteria, they must be synthesized. The carbon skeleton comes from intermediates of glycolysis and the Krebs cycle. The amino group of amino acids comes from an inorganic nitrogen source such as ammonium (NH3) (Figures 9 - 11).