# Chapter III: Carbohydrate catabolism

## 1-Generalities

Carbohydrate catabolism is the metabolic process by which cells break down carbohydrates (like glucose, fructose, and glycogen) to release energy in the form of ATP, which is used to power cellular activities.

Carbohydrates are a class of molecules that contain an aldehyde or ketone group along with multiple hydroxyl (–OH) groups. Their general formula includes carbon, hydrogen, and oxygen (Kopp et al., 2020).

Carbohydrates are important energy substrates, but they also serve many other roles:

- Structural role: for example, cellulose in plants.
- Water retention: for example, glycosaminoglycans.
- Components of other biomolecules: such as nucleotides, glycolipids, and glycoproteins.

Carbohydrates can be classified based on their complexity into monosaccharides, disaccharides, and polysaccharides (Kopp et al., 2020)

### 1. Monosaccharides (Simple Sugars)

- ✓ Definition: The simplest form of carbohydrates; single sugar units.
- ✓ Examples:
    - Glucose: Main energy source for cells.
    - Fructose: Found in fruits and honey.
    - Galactose: Part of lactose in milk.
- ✓ Characteristics: Soluble in water, sweet-tasting, and cannot be hydrolyzed into simpler sugars (Sarkar et al., 2024).

### 2. Disaccharides

- ✓ Definition: Carbohydrates composed of two monosaccharide units linked by a glycosidic bond.
- ✓ Examples:

- Sucrose: Glucose + Fructose (table sugar).
- Lactose: Glucose + Galactose (milk sugar).
- Maltose: Glucose + Glucose (formed during starch digestion).

✓ Characteristics: Sweet, soluble in water, and can be broken down into monosaccharides during digestion (Sarkar et al., 2024).

**3. Polysaccharides (Complex carbohydrates)**

✓ Definition: Long chains of monosaccharide units, often branched or unbranched.
✓ Examples and Functions:

- Starch: Plant energy storage.
- Glycogen: Animal energy storage (mainly in liver and muscles).
- Cellulose: Plant structural component, forms cell walls.
- Chitin: Structural component in fungi and exoskeletons of arthropods.

✓ Characteristics: Usually insoluble in water, not sweet, and provide structural support or long-term energy storage.

The carbohydrates in food that can be broken down by microorganisms are numerous and varied.

Some carbohydrates, particularly polymers, cannot enter the cell directly and must first be hydrolyzed by enzymes secreted into the surrounding environment. The breakdown products then enter the cell.
The transformation of carbohydrate polymers**,** sugars, and various other organic substances often leads to the formation of glucose or related molecules, and sometimes to pentoses or other derivatives (Sarkar et al., 2024).

**2-Energetic metabolism and consequences**

Microorganisms produce energy during catabolism through exergonic reactions. To prevent energy loss as heat, these exergonic (energy-releasing) reactions are coupled with endergonic reactions (energy-consuming).

In this way, the energy is either stored in ATP molecules or immediately used in reactions that require ATP (Prakasham and Kumar, 2019).

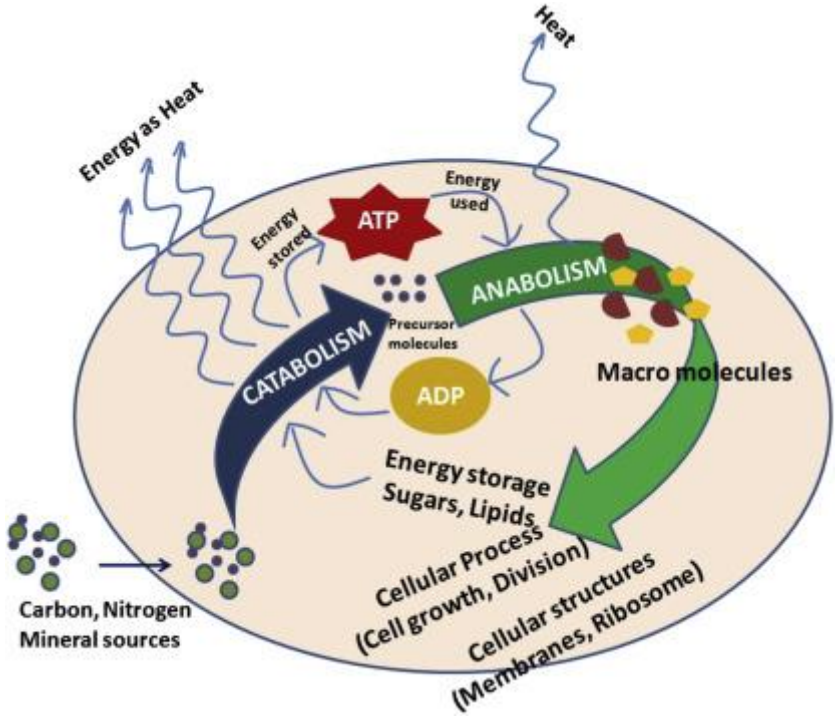



**Figure 1. Energetic metabolism in bacteria (Prakasham and Kumar, 2019)**

### 3-Energy production mechanism

The energy required by microorganisms can be obtained from:

1. **Light**: in phototrophic organisms.
2. **Oxidation of chemical substances**: in chemotrophic organisms.

In both cases, the energy is stored as biologically usable chemical bond energy in ATP molecules. The energy released from ATP breakdown is then used to drive biosynthetic (anabolic) reactions (Doelle, 1969).

### 3-1-Phototrophy

Photosynthesis, which allows plants to obtain energy from light, also occurs in green algae and some bacterial species.

The general process of photosynthesis includes two main stages:

1. Photophosphorylation (Light reaction)
    - Produces ATP and reducing power (hydrogen donors).

- This is the primary energy-generating reaction that provides usable energy for the cell.
- Often called the “light-dependent phase.”

2. Synthesis of organic compounds (Dark reaction)
    - Involves the formation of organic molecules, usually stored as carbohydrate reserves.
    - Often called the “light-independent” or “dark phase.”

Bacterial photosynthesis differs mainly from that of higher plants because it never produces free oxygen. The pigments and electron donors are also different—typically hydrogen or sulfur, but never water, as in plants (Doelle, 1969).

This type of photosynthesis results in the production of ATP from ADP and inorganic phosphate, providing energy for the bacterial cell.

Most microorganisms of interest in the food industry are chemo-organo-trophs (heterotrophs), which obtain their energy from the breakdown of organic matter. These are oxidation reactions, often involving dehydrogenation, sometimes coupled with hydration or decarboxylation:

**R-CH2OH --------> R-CHO + $2H^+$ + 2e + énergie**

In all cases, there is a loss of electrons coupled with a loss of protons. These electrons and protons are transferred—sometimes through a long series of steps—via a redox chain, ultimately reducing a final electron acceptor. During this transfer, ATP is generated, providing energy for the cell (Clark and Parikh, 2020).

The electron transport system varies depending on the microorganism and the redox potential of the initial electron donor and final electron acceptor. It involves enzymes (such as dehydrogenases and reductase cytochromes) and coenzymes (FAD, $NAD^+$, $NADP^+$, cytochromes, etc.), which act as both electron acceptors and donors.

This system is a series of coupled oxidation-reduction reactions, the classic example being the respiratory chain or oxidative phosphorylation chain in eukaryotes, primarily located in the mitochondria. In bacteria, the system is membrane-bound and exhibits many variations (Clark and Parikh, 2020).

There is a mechanism for ATP formation that does not involve an electron transport chain. In this process, a substrate-level phosphorylation occurs:

1. An organic substrate is phosphorylated using inorganic phosphate.

2. The substrate undergoes oxidation via dehydrogenation, generating a high-energy bond.
3. Finally, dephosphorylation occurs, resulting in the formation of ATP.

This is known as substrate-level phosphorylation, and it provides a direct way to produce ATP without the need for a redox chain (Clark and Parikh, 2020).

$$RH_2 + Pi \text{ ----> } R\sim P + 2H^+ + 2\,e \quad \text{Ater} \quad R\sim P + ADP \text{ ----> } R + ATP$$

**4- Respiration types**

- Completely oxidized substrate → Respiration
- Partially oxidized and incompletely degraded substrate → Fermentation

Both respiration and fermentation can be:

- Aerobic: requiring oxygen
- Anaerobic: which may be strict (obligate) or facultative (optional)

The nature of the metabolic pathway used determines the energy yield from the substrate's degradation.

The final electron and proton acceptor determines the different energetic types:

- In aerobic conditions: $O_2$ → aerobic respiration
- In anaerobic conditions:
    - Mineral acceptor → anaerobic respiration
    - Organic acceptor → fermentation (Liu et al., 2024).

**4-1- Respiration**

The respiratory chain is located at the membrane. It consists of a series of enzymes and coenzymes that ensure the transfer of electrons from a donor to an acceptor, coupled with the synthesis of ATP molecules.

All aerobic respiration mechanisms share the feature that the final electron and proton acceptor is atmospheric oxygen.

Microorganisms that rely solely on this system are obligate aerobes (Liu et al., 2024).

**a. Glycolysis**

Glycolysis consists of 10 major steps, each catalyzed by a specific enzyme (Chandel, 2021).

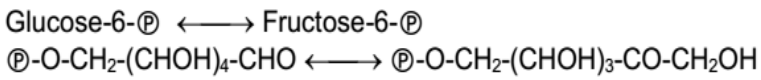
***A – Enzymatic steps of the first phase***

1. **Phosphorylation of glucose to glucose-6-phosphate (G6P)**
    - This transphosphorylation reaction converts glucose into glucose-6-phosphate.

- Catalyzed by glucokinase or hexokinase.
- Hexokinase is not glucose-specific; it can phosphorylate other sugars such as mannose or fructose.
- Consumes 1 ATP molecule.
- This reaction is irreversible.

2. **Isomerization of glucose-6-phosphate to fructose-6-phosphate**
    - Catalyzed by 6-phosphohexose isomerase, particularly phosphoglucoisomerase (PGI) for glucose.
    - This reaction is reversible.
    - It is specific for these two compounds: starting from either glucose-6-phosphate or fructose-6-phosphate always leads to the same equilibrium.

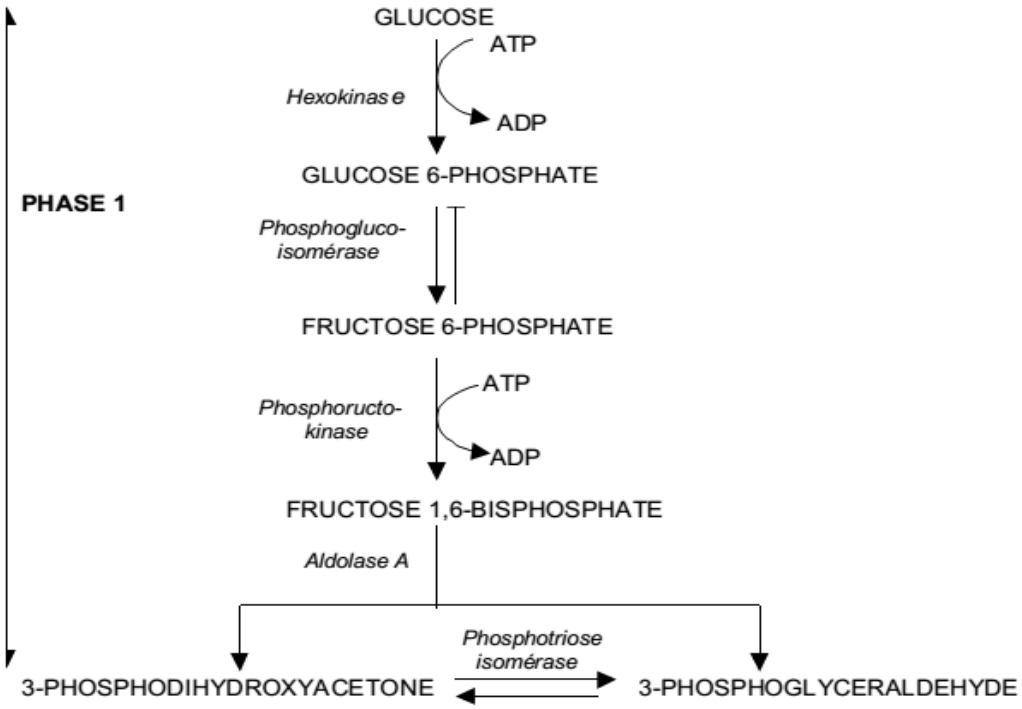
Glucose-6-phosphate is a central metabolic intermediate. Its carbon 2 is the only one with a hydroxyl group in the axial position, which facilitates its oxidation. This reaction occurs without significant change in internal energy (Chandel, 2021).




3. **Phosphorylation of fructose-6-phosphate (F6P)**
    - This reaction is catalyzed by phosphofructokinase (PFK), also called fructose-6-phosphate kinase, on carbon 1 to produce fructose-1,6-bisphosphate (F1,6BP).
    - It consumes 1 ATP molecule. ATP, in the presence of magnesium, acts as the energy and phosphate donor coenzyme.
    - PFK is the slowest enzyme of glycolysis.
    - It catalyzes the committed step of carbohydrate metabolism in energy production.
    - Therefore, it is the key regulatory enzyme that limits the overall rate of glycolysis (Chandel, 2021).
4. **Cleavage of fructose-1,6-bisphosphate into triose phosphates**
    - Fructose-1,6-bisphosphate (F1,6BP) is split into two triose-phosphate molecules by the enzyme aldolase, which is an aldolase lyase, a characteristic enzyme of this metabolic pathway.
    - Carbon allocation:
        - Carbons 1, 2, and 3 of F1,6BP form dihydroxyacetone phosphate (DHAP).
        - Carbons 4, 5, and 6 of F1,6BP form glyceraldehyde-3-phosphate (G3P).

5. **Isomerization of Triose Phosphates**
    - The two triose phosphates are isomers.
    - The conversion of the ketose (dihydroxyacetone phosphate, DHAP) into an aldose (glyceraldehyde-3-phosphate, G3P) is catalyzed by triose phosphate isomerase.
    - Similar to phosphohexose isomerase, this enzyme performs an internal oxidation-reduction between carbons 1 and 2.
    - In the continuation of glycolysis, only G3P is used, meaning DHAP is interconverted into glyceraldehyde-3-phosphate.
    - This reaction completes the first phase of glycolysis.

At the end of this phase, all hexoses are converted into two molecules of G3P, ready for the energy-generating phase of glycolysis.



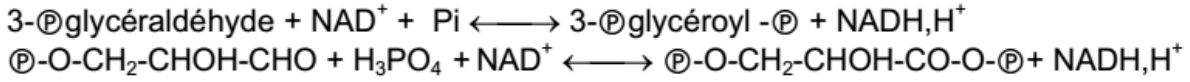

***B – Enzymatic steps of the second phase of glycolysis***

The second phase is the ATP- and pyruvate-producing phase. It includes the only oxidation-reduction reaction in glycolysis, which generates NADH + $H^+$ (Chaudhry and Varacallo, 2025).

The two glyceraldehyde-3-phosphate (G3P) molecules obtained from the first phase undergo a sequence of reactions leading to pyruvate. The following description is for one molecule of G3P (Chaudhry and Varacallo, 2025).

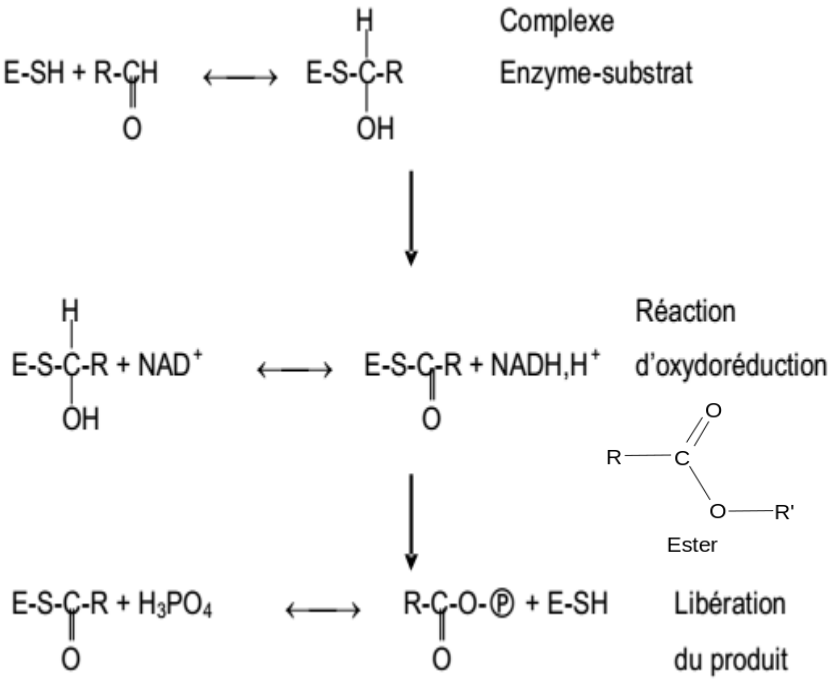
## 6. Oxidation of G3P to 1,3-bisphosphoglycerate (1,3-BPG)

- Catalyzed by glyceraldehyde-3-phosphate dehydrogenase (3-phosphoglyceraldehyde dehydrogenase).
- Requires the presence of inorganic phosphate (Pi).
- The aldehyde group of G3P is oxidized to a carboxyl group, which is then linked to phosphate via a high-energy bond.
- The product formed is 3-phosphoglyceroyl-1-phosphate (1,3-BPG) (Chaudhry and Varacallo, 2025).




The requirement for inorganic phosphate (Pi) in the previous step is explained by the catalytic mechanism of the enzyme:

- The enzyme contains a thiol group (-SH) in its active site.
- During the formation of the enzyme-substrate complex, a covalent bond is created between the substrate and the enzyme.
- According to this mechanism, the release of the product involves the formation of a carboxyphosphate ester bond, which is made possible by the high energy released during oxidation of the aldehyde group (Chaudhry and Varacallo, 2025).



7. **Phosphate transfer to ADP – ATP synthesis**
    - This reaction is catalyzed by phosphoglycerate kinase (a phosphotransferase), producing 3-phosphoglycerate (3-PG) and ATP.
    - The reaction is reversible.

Through the combined action of glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase, the reaction sequence results in the formation of ATP (Chaudhry and Varacallo, 2025).

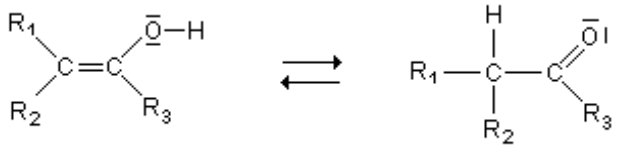
glycéraldéhyde 3-Ⓟ + $NAD^+$ + ADP + Pi ⟶ glycérate 3-Ⓟ + $NADH,H^+$ + ATP

Ⓟ-O-$CH_2$-CHOH-CHO + $NAD^+$ + ADP + Pi ⟶ Ⓟ-O-$CH_2$-CHOH-$COO^-$ + $NADH,H^+$ + ATP

8. **Isomerization of 3-phosphoglycerate to 2-phosphoglycerate**
    - The phosphate group is moved from position 3 to position 2.
    - Catalyzed by phosphoglycerate mutase (an intramolecular transfer isomerization).
    - $Mg^{2+}$ is required as a coenzyme.
    - The reaction is reversible.

glycérate 3-Ⓟ ⟷ glycérate 2-Ⓟ

9. **Dehydration of 2-phosphoglycerate to phosphoenolpyruvate (PEP)**
    - This is the second reaction that generates a high-energy phosphate bond.
    - Catalyzed by enolase (a hydratase).
    - The elimination of water causes an electronic rearrangement in the molecule.
    - Produces phosphoenolpyruvate (PEP), the highest-energy molecule formed by the cell.
    - The reaction is reversible.
10. **Phosphate transfer from phosphoenolpyruvate (PEP) to ADP – Transphosphorylation**
    - This reaction is catalyzed by pyruvate kinase (a phosphotransferase).
    - $Mg^{2+}$ or $Mn^{2+}$ is required for activity.
    - Enolpyruvate formation: PEP undergoes keto-enol tautomerization.
    - Pyruvate formation: The enol form of PEP is converted into pyruvate, completing glycolysis (Chaudhry and Varacallo, 2025).



**Inputs used in glycolysis:**

- 1 mole of glucose
- 2 moles of oxidized coenzymes ($NAD^+$)
- 4 moles of ADP
- 2 moles of ATP
- 2 moles of inorganic phosphate (Pi)

**Products formed:**

- 2 moles of pyruvate
- 2 moles of reduced coenzymes (NADH)
- 4 moles of ATP
- 2 moles of water ($H_2O$)
- 2 moles of protons ($H^+$)

**Net energy yield:**

- 2 moles of ATP are produced per mole of glucose metabolized (Chaudhry and Varacallo, 2025).

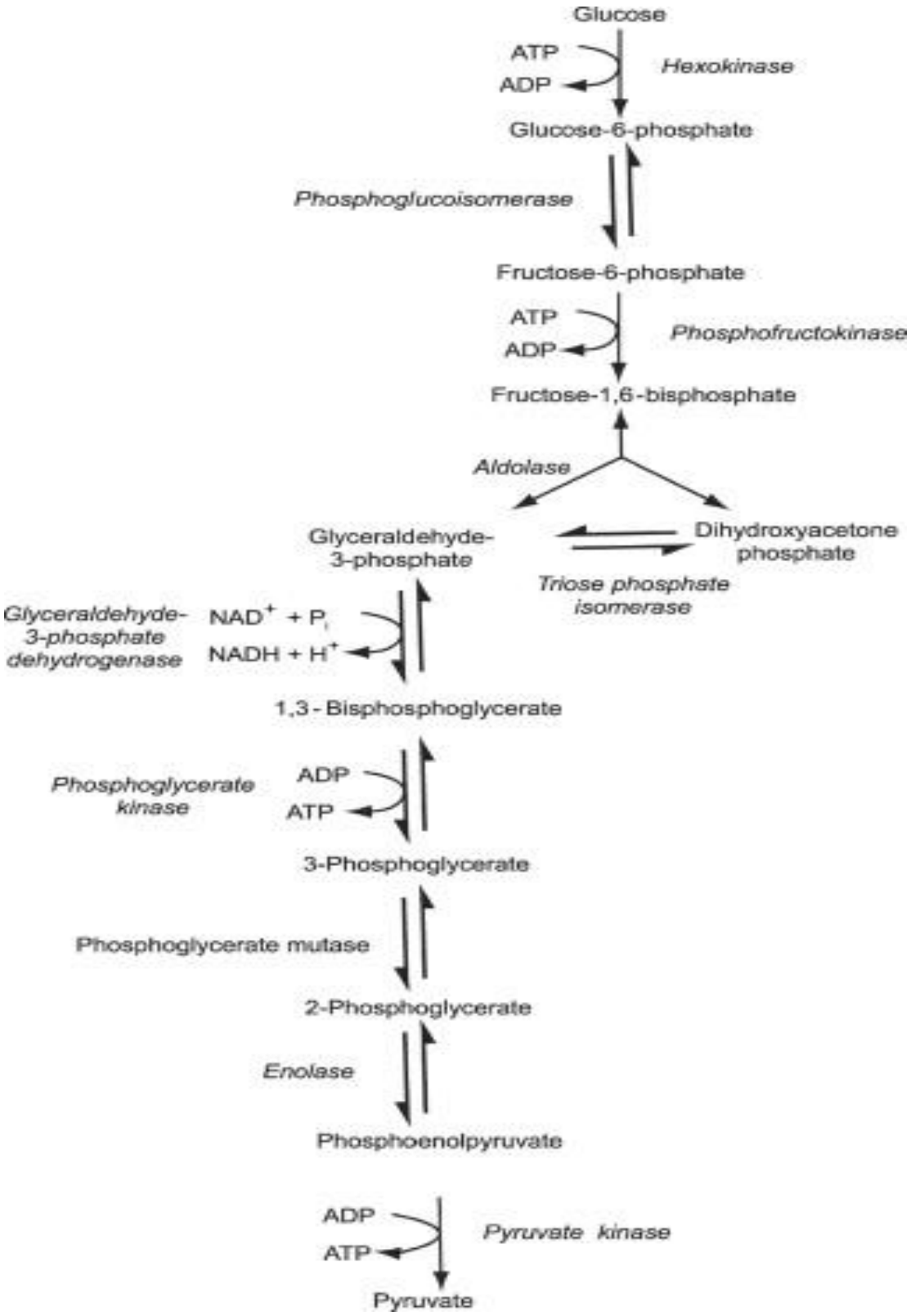



**Figure 2. Glycolysis pathway (Chan, 2003)**

✓**Regulation of glycolysis**

In metabolic pathways, enzymes that catalyze irreversible reactions are potential control points. In glycolysis, enzymes are regulated by three main mechanisms:

1. Regulation by allosteric effectors
2. Regulation by phosphorylation/dephosphorylation
3. Regulation of gene expression of these enzymes

In glycolysis, three irreversible reactions are particularly notable:

1. The phosphorylation of glucose to glucose-6-phosphate, catalyzed by glucokinase or hexokinase. Hexokinase is inhibited by glucose-6-phosphate.
2. The phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate, catalyzed by 6-phosphofructokinase (PFK). This enzyme is inhibited by ATP and citrate (in humans, by adrenaline in muscle) and activated by insulin.
3. The phosphorylation of phosphoenolpyruvate to enolpyruvate, catalyzed by pyruvate kinase. This enzyme is inhibited by pyruvate, alanine, and ATP.

Overall, it can be summarized as follows:

- Inhibition of glycolysis occurs when there is excess energy, i.e., high ATP levels.
- Activation of glycolysis occurs when there is energy deficiency, i.e., high ADP levels (Chaudhry and Varacallo, 2025).

**b. Krebs cycle (Tricarboxylic acid cycle / Citric acid cycle)**

The Krebs cycle is the cell's energy hub, continuing carbohydrate catabolism after glycolysis.

- It takes place in the mitochondrial matrix and occurs exclusively under aerobic conditions.

Roles of the Krebs cycle:

1. Substrate degradation: Converts acetyl-CoA (AcCoA) into $CO_2$, using oxygen indirectly.
2. Energy capture: Transfers hydrogen atoms and high-energy electrons to $NAD^+$ and FAD.
3. Energy production: Generates ATP for cellular use (Brody, 1999).

- ✓ **Main steps of the krebs cycle**

The cycle consists of nine major reactions involving eight key enzymes:

1. Condensation reaction

   The acetyl-CoA combines with oxaloacetate to form citrate, catalyzed by the citrate synthase enzyme. This reaction requires one molecule of $H_2O$ and releases CoA-SH.

2. Isomerization reaction

   The citrate is converted into isocitrate through an isomerization catalyzed by aconitase.

3. Dehydrogenation reaction

   The isocitrate is oxidized to oxalosuccinate by isocitrate dehydrogenase. During this reaction, $NAD^+$ is reduced to NADH + $H^+$.

4. β-Decarboxylation reaction

   The oxalosuccinate undergoes a non-oxidative β-decarboxylation to form α-ketoglutarate, with the release of one molecule of $CO_2$.

5. α-Decarboxylation and oxidation reaction

   The α-ketoglutarate is oxidatively decarboxylated to form succinyl-CoA, catalyzed by the α-ketoglutarate dehydrogenase complex.This step requires CoA-SH, releases $CO_2$, and produces NADH + $H^+$.

6. Transphosphorylation reaction

   The succinyl-CoA is converted into succinate by the enzyme succinyl-CoA synthetase (also known as succinate thiokinase**).** This step requires one molecule of inorganic phosphate (Pi) and releases CoA-SH. It also leads to the formation of GTP from GDP**,** which can be readily converted to ATP**.**

7. Dehydrogenation reaction

   The succinate is oxidized to fumarate by succinate dehydrogenase. During this process, FAD is reduced to $FADH_2$**.**

8. Hydration reaction

The fumarate is hydrated to form malate**,** catalyzed by the enzyme fumarase**.** This reaction requires one molecule of $H_2O$**.**

9. Final dehydrogenation reaction

   The malate is oxidized to regenerate oxaloacetate**,** in a reaction catalyzed by malate dehydrogenase**.**
   In this step, $NAD^+$ is reduced to NADH + $H^+$ (Brody, 1999).

✓ **Summary of the Krebs cycle**

As mentioned earlier, under aerobic conditions, acetyl-CoA enters the Krebs cycle. One complete turn of the cycle; that is, the oxidation of one molecule of acetyl-CoA; produces:

- 3 NADH + $H^+$, which can theoretically generate 3 ATP each through the electron transport chain, giving a total of 9 ATP,
- 1 $FADH_2$, which can theoretically yield 2 ATP,
- 1 ATP (or GTP) produced directly within the cycle.

Therefore, the theoretical total yield of one acetyl-CoA molecule is 12 ATP, although the actual yield is around 10 ATP due to energy losses during transfer processes (Brody, 1999).

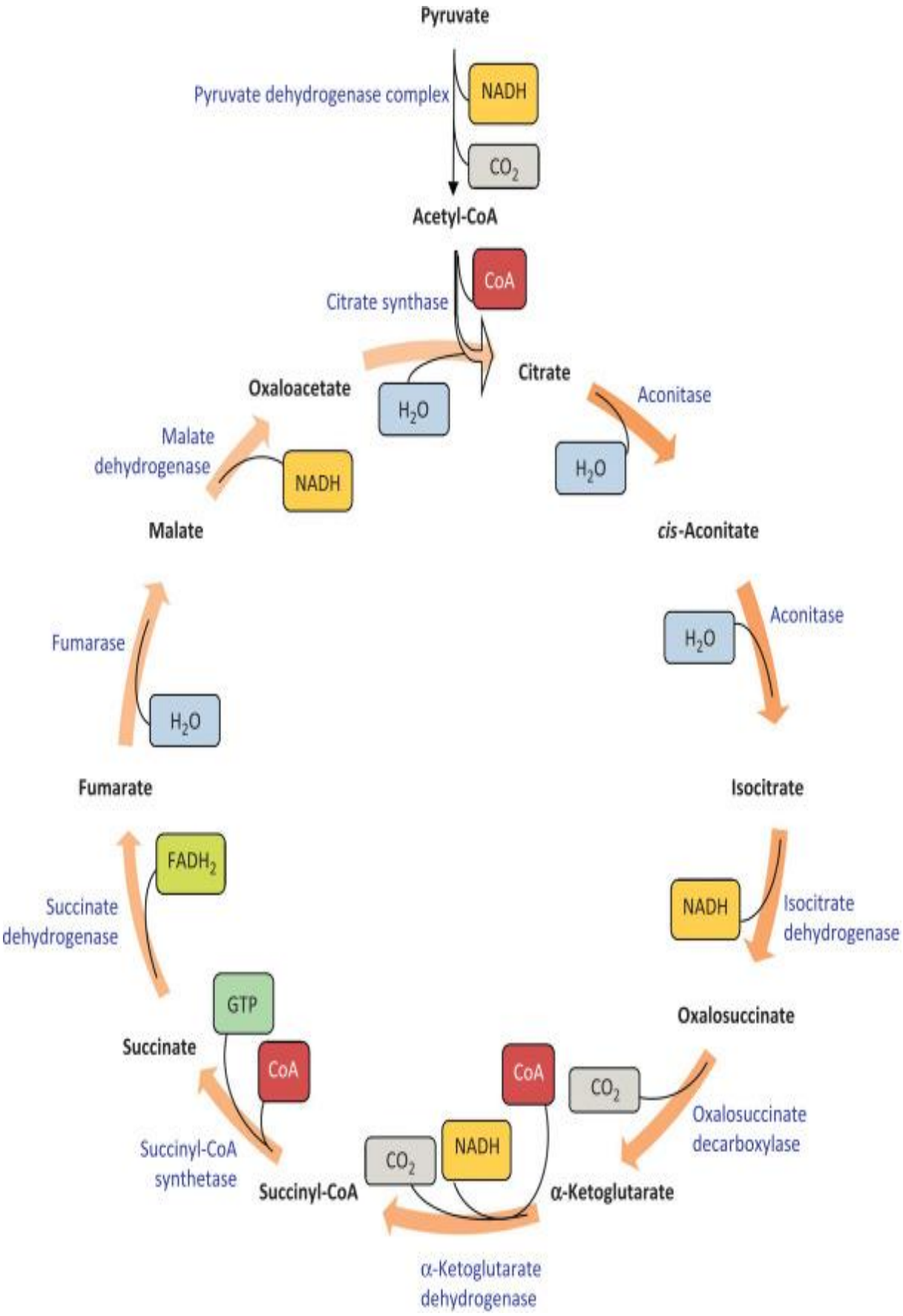



**Figure 3. Krebs cycle (Kumar and Dubey, 2019)**

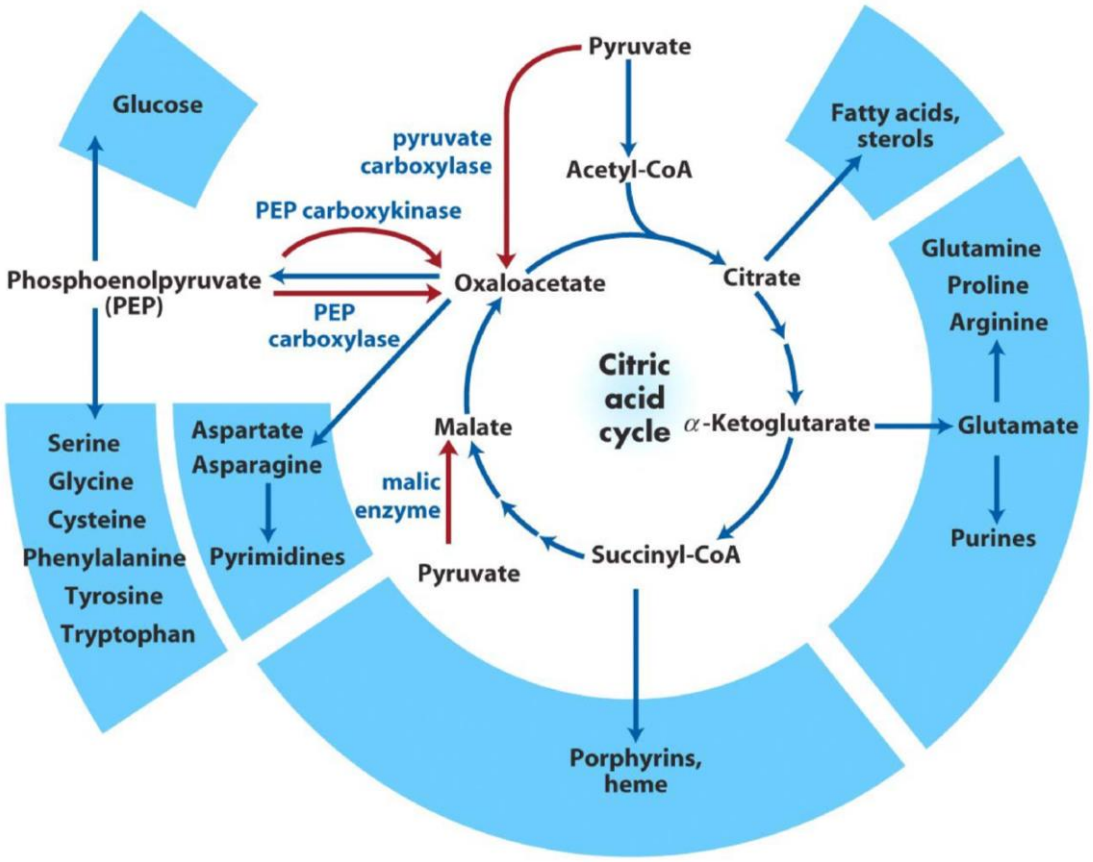



**Figure 4. Usefulness of citric acid cycle (Mailloux et al., 2020)**

### c. Electron transport chain (Respiratory chain)

**Cytochromes:**

Cytochromes are redox systems that transfer electrons but are unable to carry hydrogen. They are proteins containing a prosthetic group made of a protoporphyrin ring with a central iron atom, which plays a key role in electron transport.

**Cofactors:**

Two main cofactors participate in electron transfer:

- *Flavin adenine dinucleotide (FAD)* – derived from riboflavin (vitamin $B_2$).
- *Nicotinamide adenine dinucleotide ($NAD^+$)* – a dinucleotide composed of an adenine nucleotide linked to another whose base is nicotinamide.

These cofactors act as electron and proton carriers, transferring reducing equivalents to the components of the respiratory chain for ATP generation.

The respiratory chain corresponds to an association of protein complexes located in the inner membrane of the mitochondrion. Together with ATP synthase, these complexes are responsible for oxidative phosphorylation.

This process links the oxidation of NADH and $FADH_2$, both produced during the main catabolic pathways of the organism (glycolysis, Krebs cycle, β-oxidation of fatty acids, etc.), to the production of ATP, through the formation of a proton gradient across the membrane (Brody, 1999).

**Functioning of the electron transport chain**

Throughout the respiratory chain, the electrons originating from NADH and $FADH_2$ gradually lose energy. This released energy is used to create an electrochemical proton gradient between the intermembrane space and the mitochondrial matrix. The high-energy electrons are transferred successively through several complexes (Ley-Ngardigal and Bertolin, 2021):

- **Complex I (NADH dehydrogenase / NADH:ubiquinone oxidoreductase):**

  Receives electrons from NADH and transfers them to ubiquinone. This process drives the translocation of 4 protons ($H^+$) from the mitochondrial matrix into the intermembrane space.

- **Complex II (Succinate dehydrogenase / succinate:ubiquinone oxidoreductase):**

  Receives electrons from $FADH_2$. Unlike the other complexes, it does not pump protons across the membrane.

- **Complex III (Cytochrome $bc_1$ complex / Coenzyme Q:cytochrome c oxidoreductase):**

  Transfers electrons from reduced ubiquinone ($QH_2$) to cytochrome c and pumps 4 protons into the intermembrane space.

- **Complex IV (Cytochrome c oxidase):**

  Accepts electrons from cytochrome c and transfers them to molecular oxygen ($O_2$), reducing it to water ($H_2O$).

  This step is coupled with the pumping of 2 protons from the matrix to the intermembrane space (Ley-Ngardigal and Bertolin, 2021).

Two mobile electron carriers ensure transitions between the complexes:

- **Ubiquinone (Coenzyme Q):** Transfers electrons from **Complex I or II** to **Complex III**. It can also accept electrons coming from cytosolic sources.
- **Cytochrome c:** Transfers electrons between **Complex III** and **Complex IV** (Ley-Ngardigal and Bertolin, 2021).

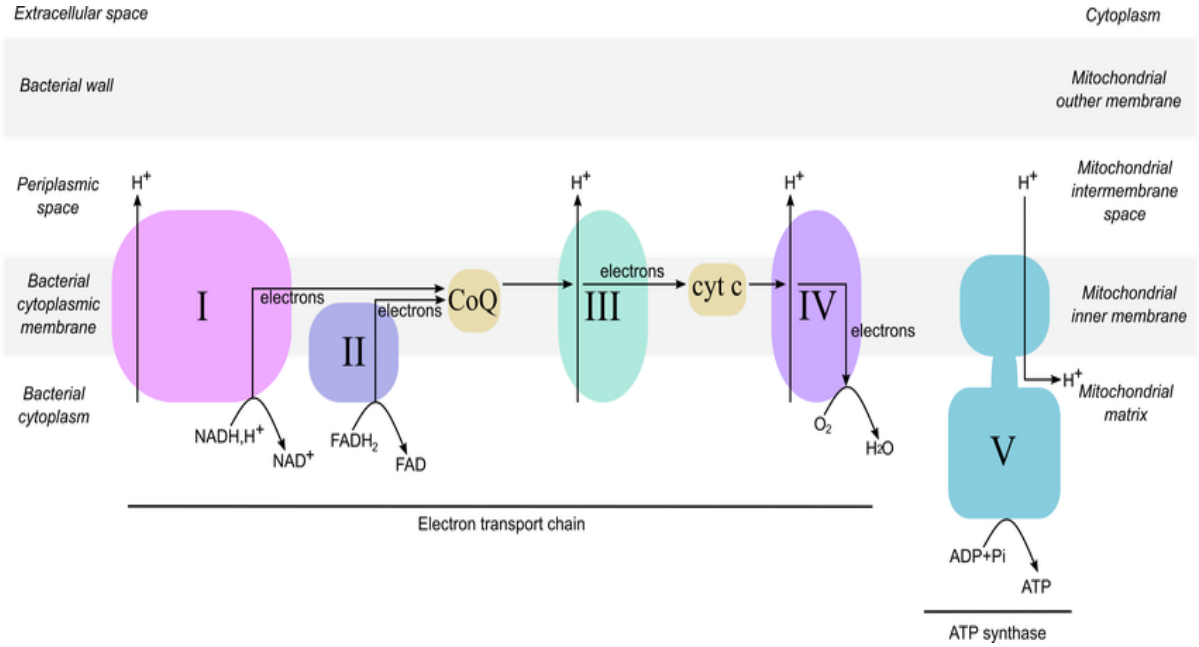



**Figure 5. Respiratory chain (Ley-Ngardigal and Bertolin, 2021)**

***The respiratory chain = dehydrogenase coenzymes + quinones + cytochromes.***

✓ **Transfer of electrons and protons**

Within the respiratory chain, electrons and protons are transferred step by step through a series of redox reactions. The electrons, derived mainly from NADH and $FADH_2$, move through the complexes and mobile carriers (ubiquinone and cytochrome c) toward the final electron acceptor, oxygen ($O_2$), which is reduced to water ($H_2O$).

During this process, the energy released from electron transfer is used to pump protons ($H^+$) from the mitochondrial matrix into the intermembrane space, creating an electrochemical gradient known as the proton motive force.

This proton gradient represents stored potential energy, which will later drive ATP synthesis through the enzyme ATP synthase (Ley-Ngardigal and Bertolin, 2021).

Unlike eukaryotes, whose electron transport chains are highly conserved, bacteria possess a wide variety of electron transfer enzymes, which can utilize a diverse range of substrates.

However, prokaryotic electron transport shares a fundamental principle with eukaryotes: the energy released during substrate oxidation is used to pump ions across a membrane, thereby generating an electrochemical gradient across the membrane.

The main difference between the respiratory chain of prokaryotes and that of eukaryotes is that prokaryotes can use a wide variety of substances as electron donors and acceptors. This flexibility allows prokaryotes to grow under very diverse environmental conditions (Ley-Ngardigal and Bertolin, 2021).

The cytochrome pathway is the most classical route (found in eukaryotic mitochondria as well as in bacteria):

- It involves a cytochrome oxidase and results in the formation of $H_2O$.
- It is usually associated with the complete degradation of the substrate.
- It is highly energetic, producing approximately 3 ATP per 2 $H^+$ / 2 electrons transferred.

The presence of flavin oxidases, which function with molecular oxygen, and superoxide dismutase (peroxidase) can lead to the production of hydrogen peroxide ($H_2O_2$) (Lu and Imlay, 2021).

**flavine oxydase**

$$\mathbf{RH_2 + O_2 \text{ ----------> } RH + O_2 + H^+}$$

**superoxyde dismutase**

$$\mathbf{2\ O_2^- + 2\ H^+ \text{ ----------> } H_2O_2 + O_2}$$

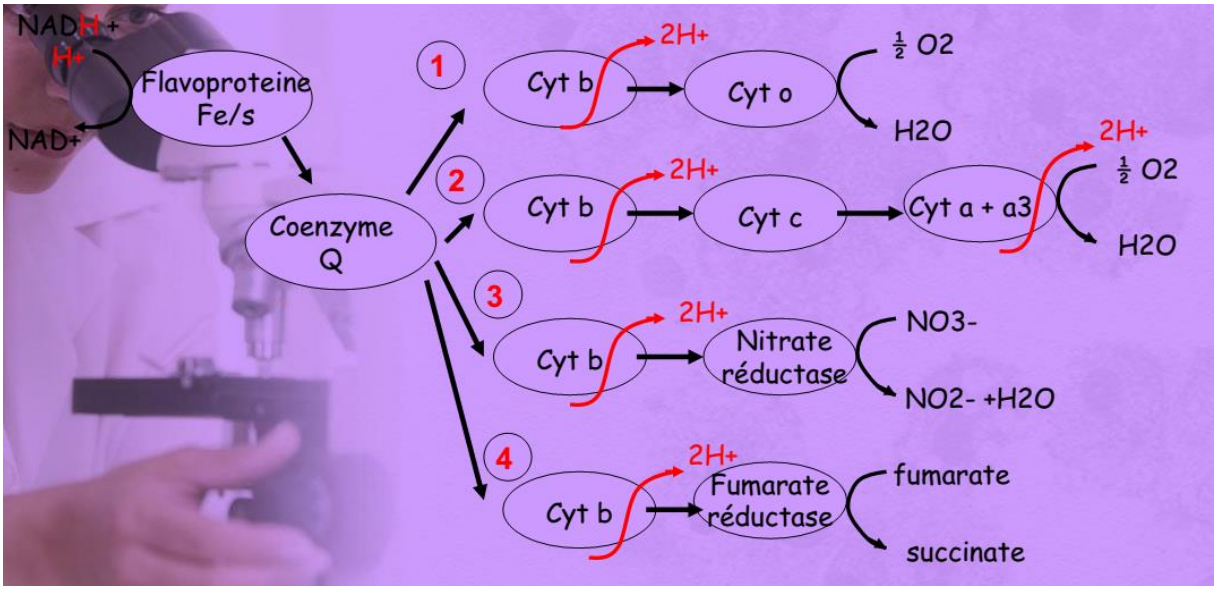
This peroxide is toxic to the cell unless it possesses catalase, an enzyme capable of breaking it down. When a microorganism has this system but lacks catalase, contact with atmospheric oxygen is toxic, making the organism anaerobic.

Respiration is possible under anaerobic conditions when an oxidized mineral substance is present in the medium to serve as the final acceptor of electrons and protons (Lu and Imlay, 2021).

For example:

$$\mathbf{NO_3^- \rightarrow NO_2^- (\rightarrow N_2)}$$

Oxygenated inorganic substrates such as nitrates (nitrate respiration), sulfates (sulfate respiration), and others can act as electron acceptors.



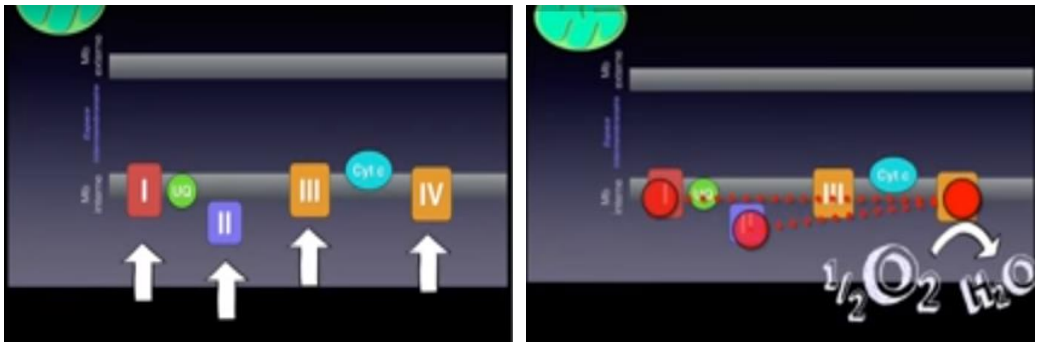

### d. Oxidative phosphorylation

Oxidative phosphorylation consists of a series of coupled redox and phosphorylation reactions (ATP synthesis), in which the energy is released step by step via an electron transport chain. The transmembrane movement of protons, accompanying the flow of electrons along the respiratory chain, generates a proton gradient across the membrane (Cook et al., 2014).

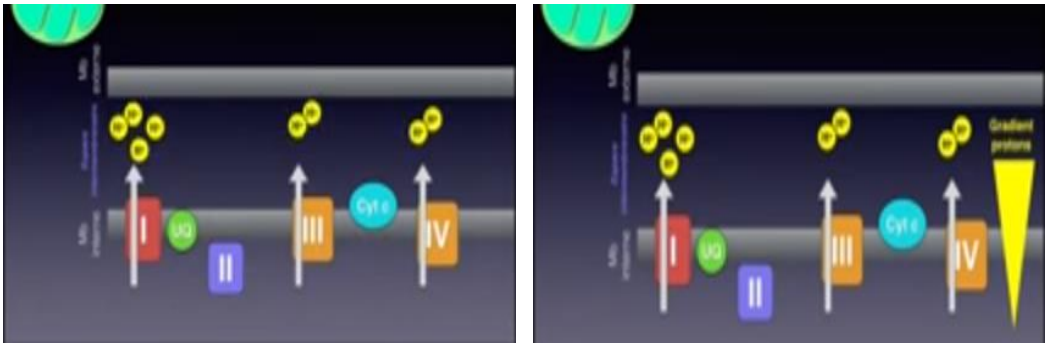
ATP synthesis occurs when $H^+$ ions pass through an ion channel within ATP synthase, moving against the gradient.

The transfer of $H^+$ from the intermembrane space into the matrix activates ATP synthase, leading to the formation of ATP.

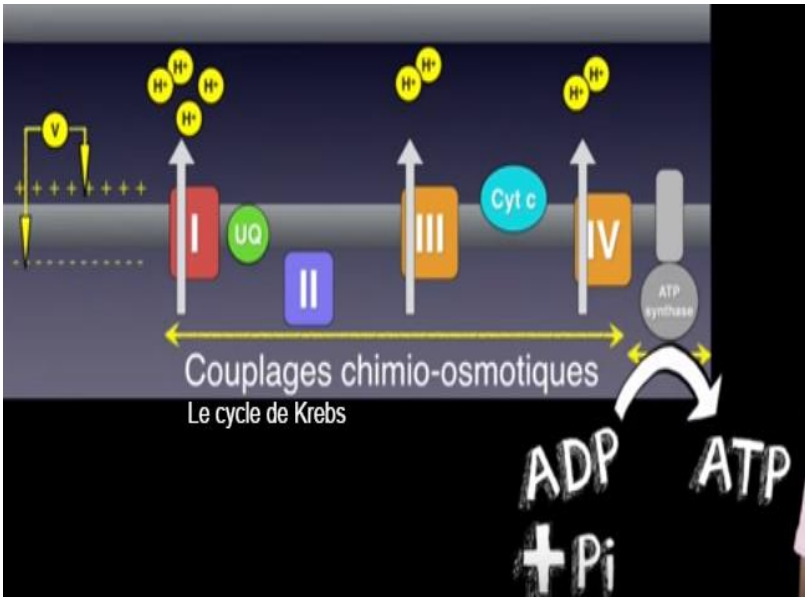
In eukaryotes, the inner mitochondrial membrane contains four macromolecular complexes that transfer electrons to the final electron acceptor, $O_2$ (Cook et al., 2014).



The reactions that take place generate free energy, which drives the transfer of protons into the intermembrane space. This process establishes a transmembrane electrochemical proton gradient.




In summary, energy transfer relies on the existence of a proton gradient. The energy is initially stored as osmotic pressure. After ATP synthase operates, the energy from the gradient is converted into chemical energy in the form of ATP. The ATP produced by this process results from chemiosmotic coupling.



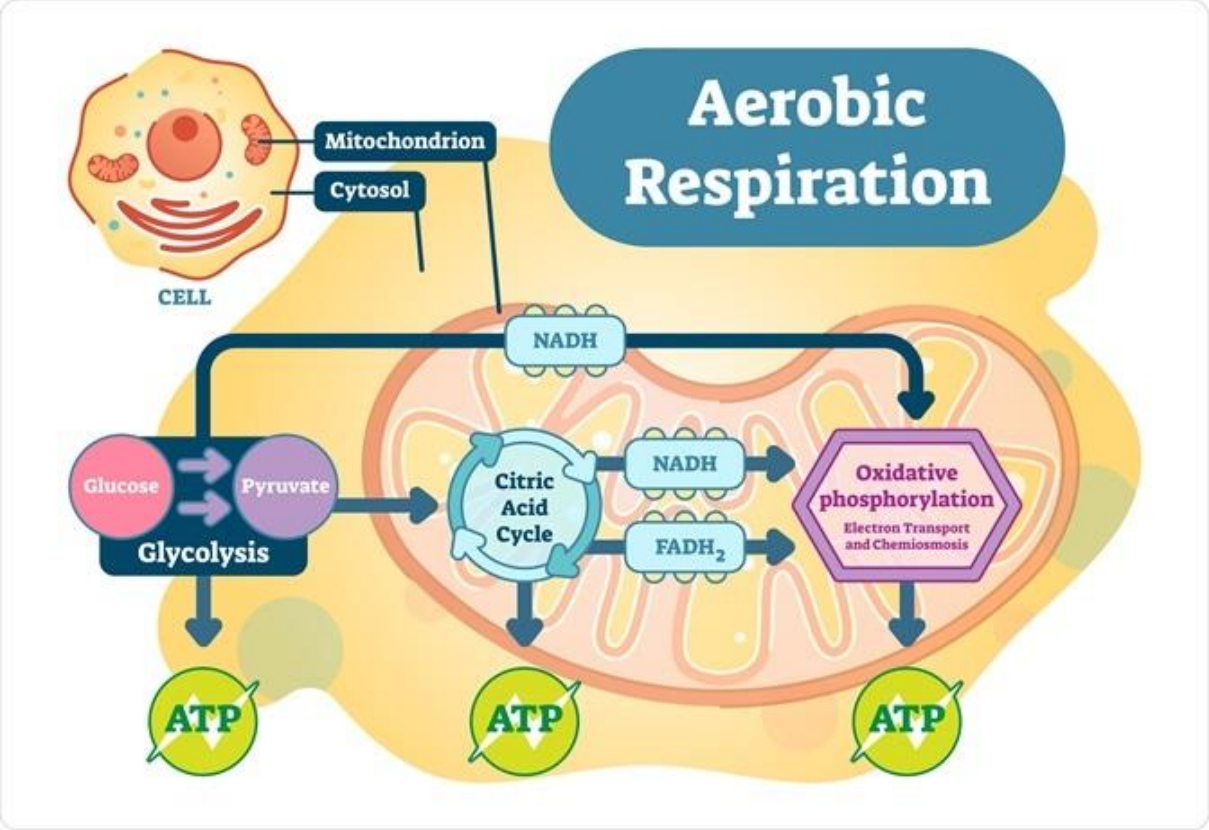



**Figure 6. Aerobic respiration and the process of glycolysis (Shutterstock.com)**

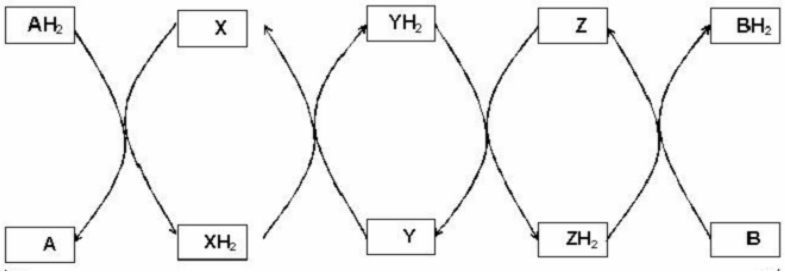



**Figure 7. Schematic representation of the respiratory chain in bacteria**

**$AH_2$:** Electron donor (energy substrate)
**A:** Oxidized donor
**B:** Final electron acceptor

The intermediate carriers (**X, Y, and Z**) can be **coenzymes** such as **NAD, FAD, FMN**, or **cytochromes**.

### 4-2- Fermentation

Fermentation was defined by Pasteur as "life without air." It is the set of biochemical reactions that provide energy to the organism through phosphorylations not coupled to membrane processes (Müller, 2008).

Fermentative pathways take place in the bacterial cytoplasm. Energy is produced by substrate-level phosphorylation, and the overall energy yield is low. Energy production through fermentation is impossible in strictly aerobic bacteria. Under aerobic conditions, only facultative anaerobic, aerotolerant bacteria can produce energy via fermentation (Müller, 2008).

In strict anaerobes and facultative anaerobes, fermentative pathways are repressed in the presence of oxygen (oxygen has an inhibitory effect).

The energy produced by fermentation is much lower than that obtained through respiration.

For example, the complete oxidation of glucose to $CO_2$ and $H_2O$ by aerobic respiration releases 674 kcal, whereas its fermentation to lactic acid produces only 22.5 kcal.

This explains the lower growth yield under anaerobic conditions compared to the growth obtained during respiratory processes (Müller, 2008).

#### a. Glucose fermentation

Glucose fermentation occurs in two main stages:

- A first series of reactions leading to the conversion of glucose into an intermediate compound, pyruvate (pyruvic acid).
- A second series of reactions involving the reduction of pyruvate, which differentiates fermentative bacteria because it produces various end products, either unique or, more often, in mixtures.

Fermentative pathways are classified according to the nature of the final fermentation products (Müller, 2008).

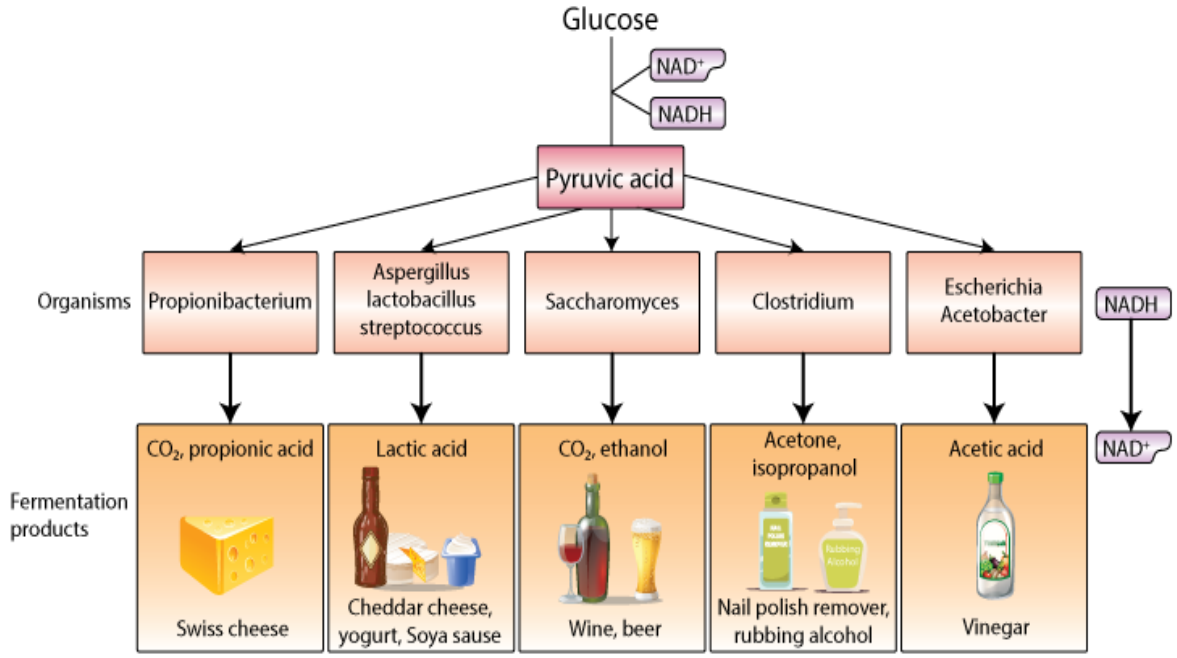



**Figure 8. Different types of fermentation (byjus.com)**

The final electron (and proton) acceptor is an organic compound. This substance can be of exogenous origin (from the external environment) or endogenous origin (produced by catabolism).

There is no net change in the overall redox potential between substrates and products (oxidation power or degree of reduction remains the same).

Example:

**Homolactic fermentation**

$$\text{Glucose} \rightarrow 2\ (2\ H^+, 2\ e^-) + 2\ \text{pyruvate}$$
$$2\ (\text{pyruvate} + 2\ H^+, 2\ e^-) \rightarrow 2\ \text{lactic acid}$$

**Alcoholic fermentation**

$$\text{Glucose} \rightarrow 2\ (2\ H^+, 2\ e^-) + 2\ \text{pyruvate}$$
$$2\ \text{pyruvate} \rightarrow 2\ CO_2 + 2\ \text{acetaldehyde}$$
$$2\ (\text{acetaldehyde} + 2\ H^+, 2\ e^-) \rightarrow 2\ \text{ethanol}$$

Like the previous examples, many fermentations can occur under anaerobic conditions, because all the released protons are used to reduce an organic metabolite.

Other fermentations must occur under aerobic conditions, because at least some of the released protons are used to reduce $O_2$ (Müller, 2008).

### b. Alcoholic fermentation

Alcoholic fermentation is very common in yeasts, particularly *Saccharomyces*. Bacteria capable of performing alcoholic fermentation are few, such as *Zymomonas mobilis*. This fermentation is involved in the production of various alcoholic beverages (wine, beer, etc.).

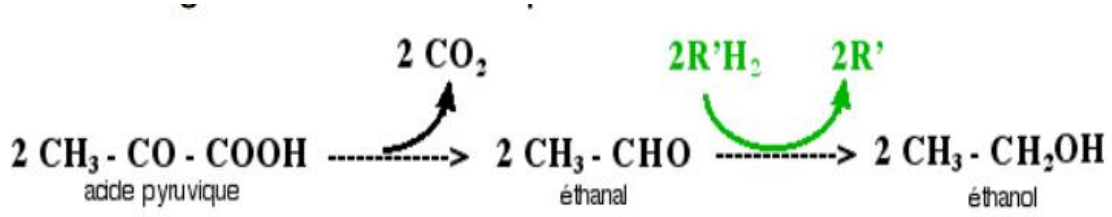
Glycolysis constitutes the first major step of alcoholic fermentation. In the case of *Zymomonas mobilis*, glucose is degraded through the Entner-Doudoroff pathway.

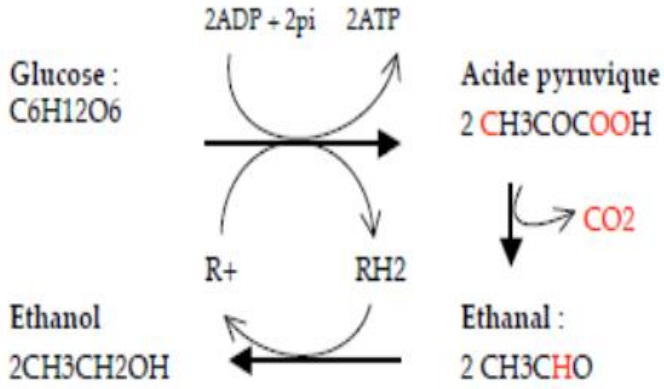
Both pathways lead to pyruvate, which is then decarboxylated to acetaldehyde (ethanal) and $CO_2$. The reduction of acetaldehyde results in the formation of ethanol.

Other substances can be produced in small amounts, particularly glycerol and acetic acid.

The conversion of one molecule of glucose into ethanol by yeasts results in the synthesis of 2 ATP molecules.

In the presence of sulfite, acetaldehyde is trapped as a bisulfite complex and cannot be reduced (Dashko et al., 2014).




### c. Lactic fermentation

Lactic fermentation is an important step in the production of cheeses, as well as in many fermented products (silages, olives, etc.) and cured meat products (sausages, ham, etc.).

In addition to their organoleptic role, lactic fermentations contribute to food stabilization and have a positive impact on food quality. In cheesemaking, acidification participates in coagulation (Abdel-Rahman et al., 2013).

- ✓ **Homolactic fermentation**

Lactic acid is the main product (95% or more) and is formed by the reduction of pyruvic acid catalyzed by lactate dehydrogenase.

This fermentation is carried out by all members of the genera *Lactococcus, Pediococcus*, and some *Lactobacillus*, as well as certain *Bacillus* species and some molds.

Lactic acid can exist in D-, L-, or DL-forms, depending on the stereospecificity of lactate dehydrogenase. A microorganism may possess an L-lactate dehydrogenase, a D-lactate dehydrogenase, or both (Abdel-Rahman et al., 2013).

- ✓ **Bacterial heterolactic fermentation**

This pathway, called also Phosphoketolase pathway, is found in *Leuconostoc* and heterofermentative *Lactobacillus.*

It involves steps of the hexose monophosphate pathway. This pathway is facultatively anaerobic and produces $CO_2$. The pathway leads to xylose-5-phosphate, which is then cleaved by the characteristic enzyme phosphoketolase into acetyl-phosphate and triose-phosphate (C3).

Compared to the EMP (Embden-Meyerhof-Parnas) pathway, this pathway is characterized by the absence of fructose-1,6-bisphosphate aldolase (which splits fructose-1,6-bisphosphate into dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate) and by the presence of phosphoketolase activity on ribulose-5-phosphate (C5). This reaction produces glyceraldehyde-3-phosphate (C3) and acetyl-phosphate (C2), both compounds with high-energy phosphate bonds.

Following two reactions involving the reduction of $NAD^+$ to NADH, this pathway is the source of the high-energy compounds characteristic of this fermentation, through a remarkable phosphorylating reaction (Pessione et al., 2010).

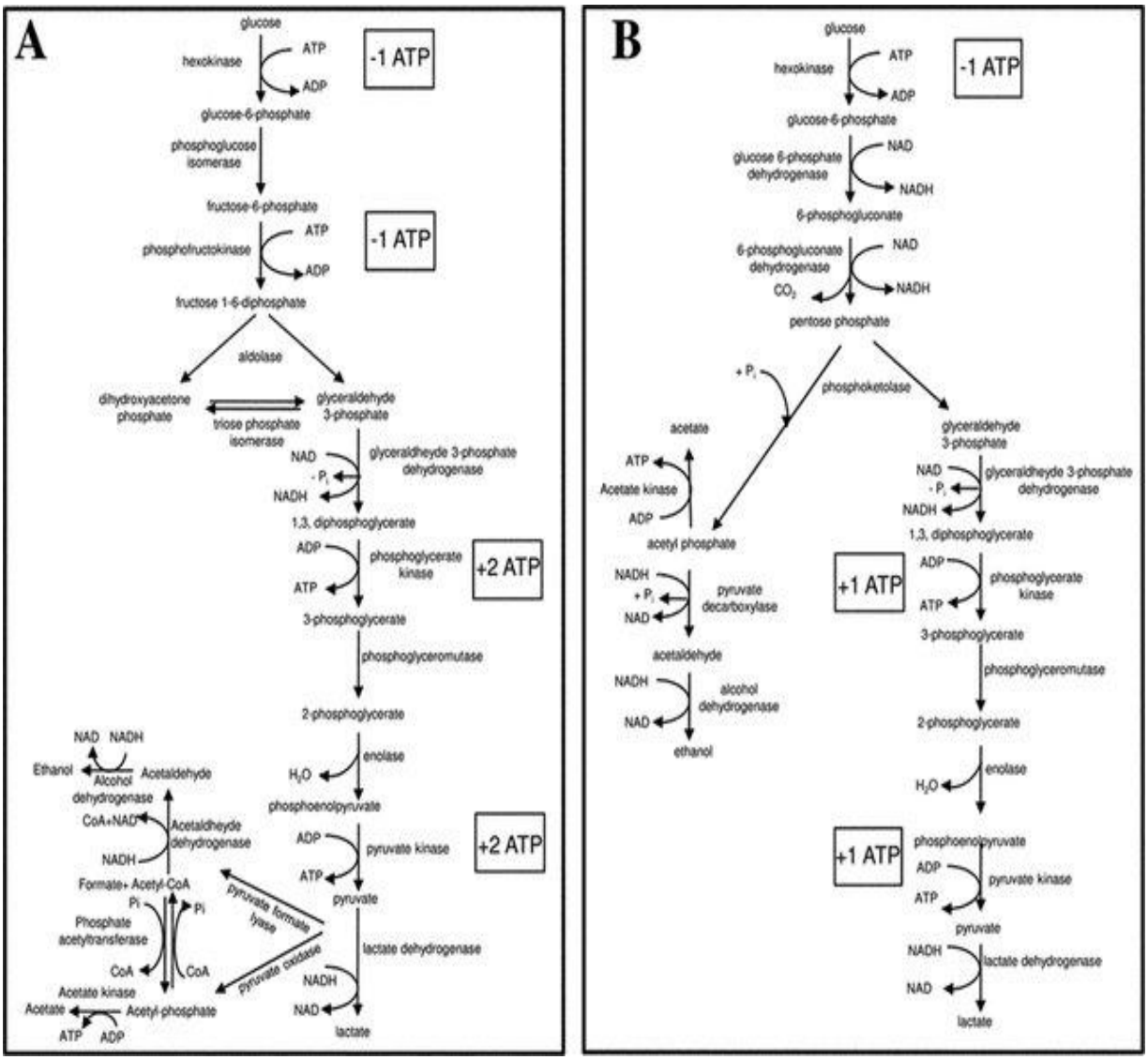



**Figure 9. Homolactic (A) and heterolactic (B) fermentation (Pessione et al., 2010)**

***How is NADH recycled in heterofermentative lactic bacteria?***

- By the classical lactate dehydrogenase reaction, which reduces pyruvate to lactate.
- Through the following series of reactions:

**acetyl-phosphate + CoA-SH → acetyl-CoA + phosphate**

**acetyl-CoA + NADH + $H^+$ → acetaldehyde + $NAD^+$**

**acetaldehyde + NADH + $H^+$ → ethanol + $NAD^+$**

Classically, to balance the pathway between oxidation and reduction at the $NAD^+$/NADH level, it is often simplified as if all acetyl-phosphate goes to ethanol. However, in nature, the situation is more complex because biosynthetic reactions consume reducing power, and a

significant fraction of acetyl-phosphate can be converted to acetate via substrate-level phosphorylation, generating ATP.

The main terminal fermentation products are therefore $CO_2$, lactate, ethanol, and acetate, with lactate predominating over ethanol and acetate (Pessione et al., 2010).

- ✓ **Fungal heterolactic fermentation**

Among molds, *Rhizopus oryzae* represents a special case.

When cultivated under aerobic conditions, it produces a mixture of lactic acid and $CO_2$, whereas under anaerobic conditions, it produces a mixture of lactic acid, ethanol, and $CO_2$.

These products are identical to those obtained during heterolactic fermentation in *Leuconostoc,* but the mechanism of formation is different: glucose degradation occurs via the glycolytic pathway.

- Under aerobic conditions, part of the pyruvate is converted into lactic acid, while the other part is oxidized ($CO_2$ + acetyl).
- Under anaerobic conditions, part of the pyruvate is converted into ethanol and $CO_2$, while the other part is converted into lactic acid (Nout, 1995).

**d. Mixed acid and butylene-glycol fermentation**

- ✓ **Mixed acid fermentation**

This pathway is carried out by Enterobacteria belonging to the genera *Escherichia, Salmonella, Proteus, Shigella, Yersinia*. It is also found in Vibrio and some Aeromonas species.

This fermentation is characterized by the production of ethanol and several organic acids, including lactic, acetic, succinic, and formic acids.

Some species (*Escherichia coli, Proteus,* and certain *Salmonella*) are highly gas-producing, possessing formic hydrogen lyase, which immediately decomposes formic acid into $H_2$ and $CO_2$ at neutral or acidic pH (Ciani et al., 2008).

- ✓ **Butylene-glycol fermentation**

Butylene-glycol fermentation is carried out by members of the genera *Enterobacter, Klebsiella*, *Serratia* (Enterobacteria), as well as by some *Aeromonas* and *Bacillus species*.

It leads to the formation of 2,3-butanediol (or 2,3-butylene glycol), the most abundant product. 2,3-butanediol is formed by the reduction of acetylmethylcarbinol (acetoin), which is produced from pyruvate via acetolactate. Acetoin and diacetyl are formed under aerobic conditions.

In general, acids are produced in small amounts, although *Serratia* produces a significant quantity of formic acid. In other Enterobacteria performing butylene-glycol fermentation, the presence of formic hydrogen lyase results in the formation of $H_2$ and $CO_2$, with $CO_2$ being more abundant than $H_2$ because it is also generated during the synthesis of 2,3-butanediol (Ciani et al., 2008).

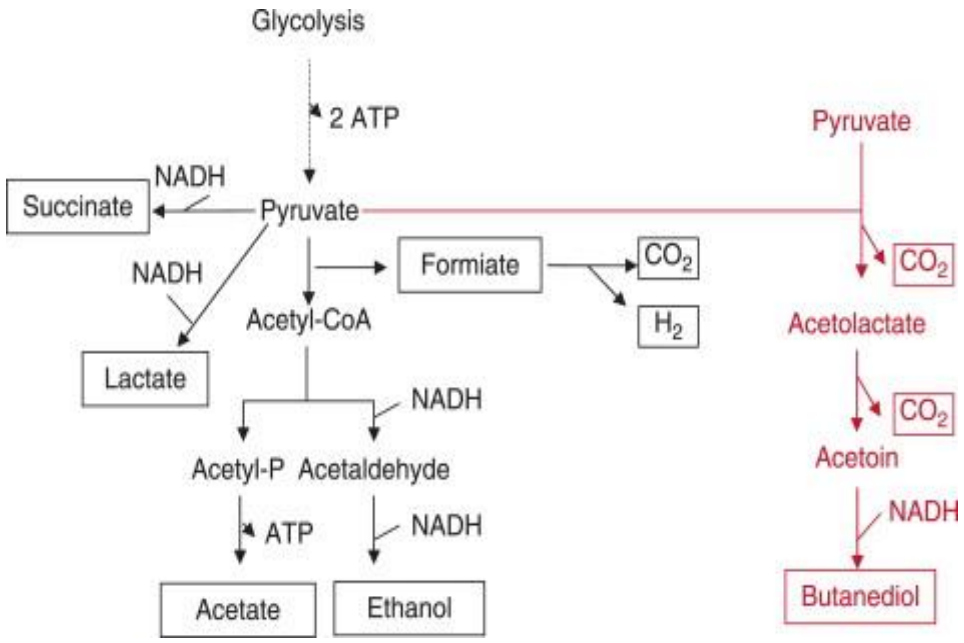



**Figure 10. End products of mixed-acid and butanediol fermentation (Ciani et al., 2008).**

### e. Butyric fermentation

Some anaerobic bacteria (*Clostridium, Butyribacterium,* and some *Serratia*) produce butyric acid, as well as acetic acid, $CO_2$, and hydrogen.

In addition to the products of butyric fermentation, some Clostridium species can also produce alcohols (Guiraud, 1999).

Pyruvate + CoA ⟶ acétyl-CoA + $Co_2$ + $H_2$

Acétyl-CoA ⟶ acétate ou acétoacétate-CoA

Acétoacétate-CoA ⟶ butenoyl-CoA ($CH_3$-CH=CH-CO-S-CoA)

Butenoyl-CoA ⟶ butyryl-CoA ($CH_3$-$CH_2$-$CH_2$-CO-S-CoA) ⟶ ac.butyrique

### f. Propionic fermentations

Various strictly or facultatively anaerobic bacteria (*Propionibacterium,* certain *Clostridium, Corynebacterium, Neisseria,* etc.) carry out fermentation producing propionic acid, acetic acid, $CO_2$, and succinic acid.

Propionic acid is generated from pyruvate through a decarboxylation of succinic acid.

Propionic fermentation can also proceed from lactate, using pyruvate as an intermediate, except in *Clostridium propionicum*, where acrylic acid serves as the intermediate.

*Propionibacterium* plays a key role in the ruminant digestive system and is also involved in the production of cooked cheeses (Ciani et al., 2013).

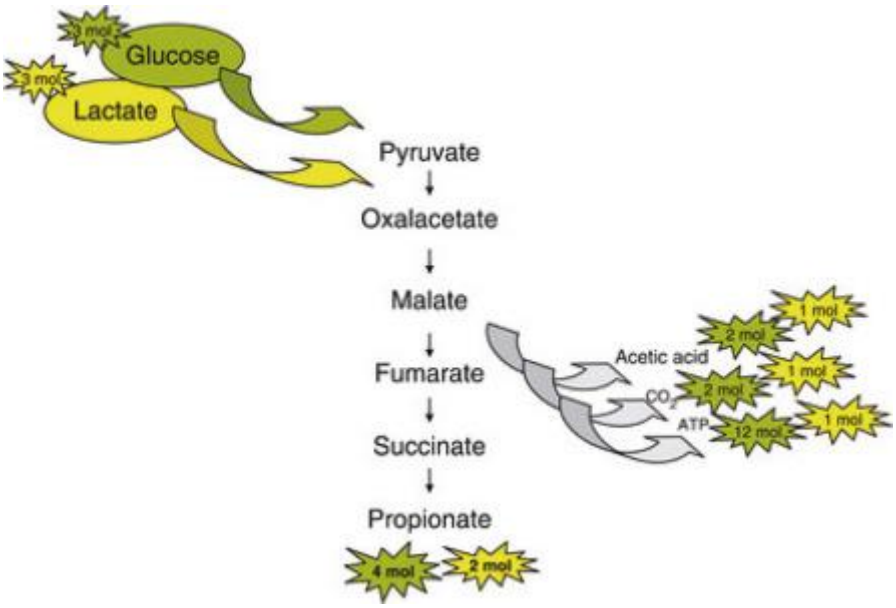



**Figure 11. Propionic acid fermentation (Ciani et al., 2013)**

### g. Oxidative fermentations

Some transformations use oxygen as the sole final acceptor of electrons and protons and are called fermentations because of the incomplete degradation of the substrate, in which the carbon is not fully converted into $CO_2$.

These processes are in fact “incomplete respirations”, where the fermentation product is more oxidized than the substrate, hence the term “oxidative fermentations.” This applies to gluconic, citric, acetic, and other fermentations (Liu et al., 2024).

### 4-3- Alternative glycolysis pathways

Besides the classical Embden-Meyerhof-Parnas (EMP) pathway, several microorganisms use alternative routes to catabolize glucose. These pathways differ in their intermediates, enzymes, and energy yields, allowing metabolic flexibility depending on environmental conditions and the organism's physiology.

#### a. Entner–Doudoroff (ED) pathway or 2-keto-3-desoxy-6-phosphogluconate

This pathway shares several steps in common with glycolysis. It was discovered by Entner and Doudoroff while studying the oxidation of glucose by *Pseudomonas* species. The Entner–Doudoroff pathway occurs mainly in aerobic microorganisms such as *Pseudomonas*, and more rarely in anaerobic species, for instance *Zymomonas mobilis*, which employs this pathway for the fermentation of glucose into ethanol.

It is also found in Azotobacter and in certain fungi.

The pathway leads to the formation of pyruvate, which serves as a starting point for other metabolic routes, including the citric acid cycle and various fermentative processes.

This pathway is found in some Gram-negative bacteria such as *Pseudomonas*, *Zymomonas*, and *Agrobacterium*. It begins with glucose-6-phosphate, which is oxidized to 6-phosphogluconate and then cleaved into pyruvate and glyceraldehyde-3-phosphate. The overall reaction produces 1 ATP, 1 NADH, and 1 NADPH per molecule of glucose, which is less efficient than the EMP pathway but provides reducing power for biosynthesis (Bender, 2013).

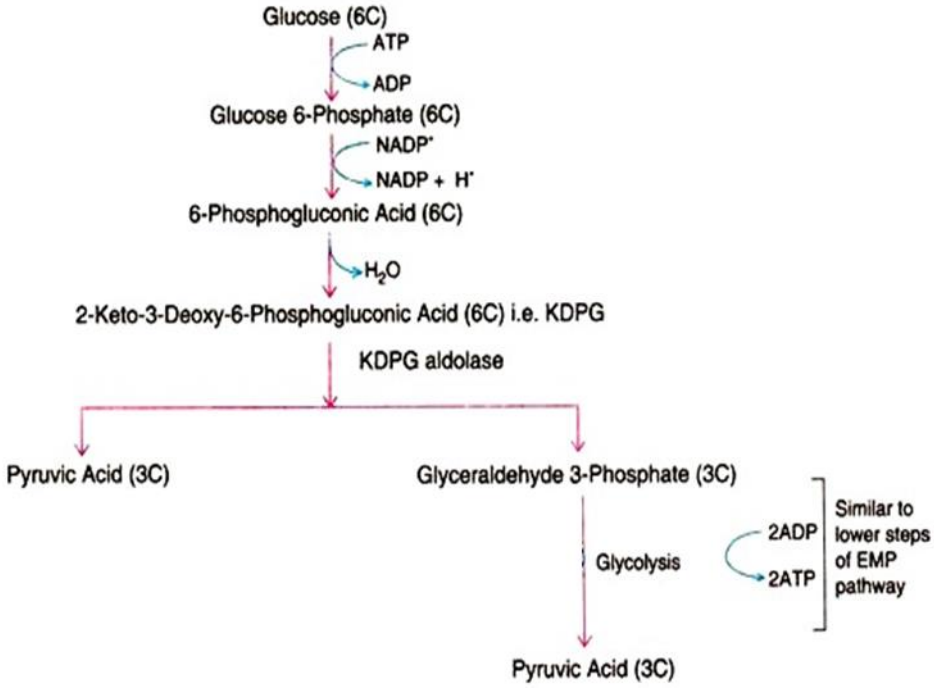



**Figure 12. Entner–Doudoroff pathway (Gupta and Gupta, 2021)**

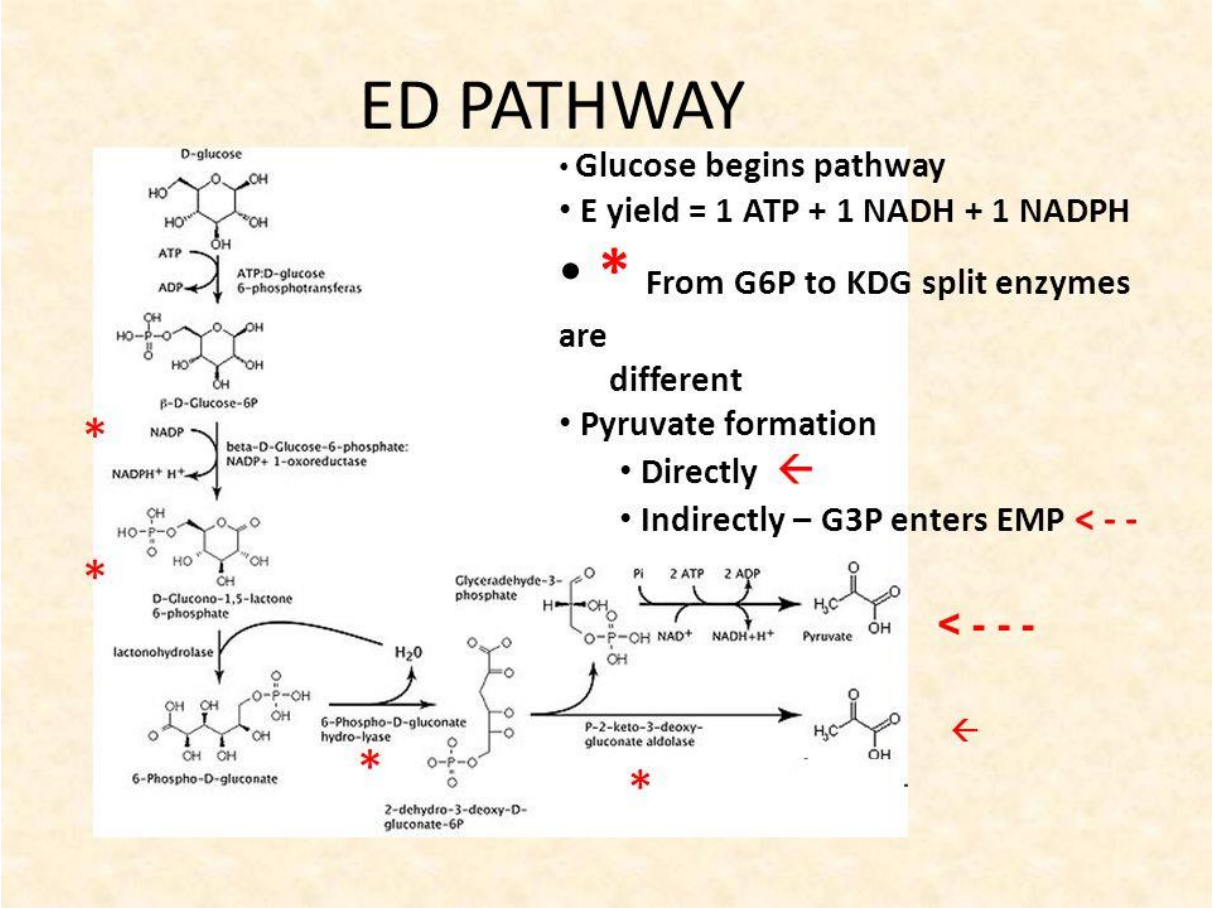
✓ **Main steps of the Entner–Doudoroff pathway**

The essential steps of this pathway are as follows:

- **Activation of glucose** by ATP through the action of *glucokinase*.
- **Oxidation of the aldehyde group** of glucose-6-phosphate, catalyzed by *glucose-6-phosphate dehydrogenase*, leading to the formation of *6-phosphogluconate* and the concurrent reduction of $NADP^+$ to NADPH.
- **Dehydration of 6-phosphogluconate** by *phosphogluconate dehydratase*, producing *2-keto-3-deoxy-6-phosphogluconate* (KDPG).
- **Cleavage of KDPG** by *KDPG aldolase*, yielding one molecule each of *glyceraldehyde-3-phosphate (G3P)* and *pyruvate*.
- **Conversion of G3P into pyruvate** through the glycolytic sequence, generating *2 molecules of ATP* and *1 molecule of NADH* per triose phosphate.

Overall, for each molecule of glucose metabolized through this pathway, the yield is 1 ATP, 1 NADPH, and 1 NADH.

Nicotinamide adenine dinucleotide phosphate (NADP) is a redox cofactor structurally similar to nicotinamide adenine dinucleotide (NAD). The main difference between the two molecules lies in the presence of a phosphate group attached to the second carbon of the β-D-ribofuranose moiety of the adenosine residue (Bender, 2013).



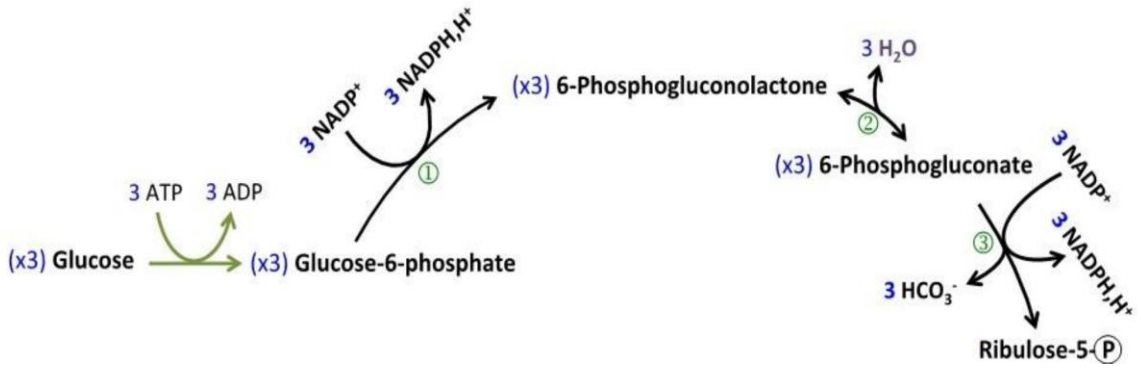

**b. Pentose phosphate pathway**

This aerobic pathway is highly significant because it provides pentose sugars, which are essential for the synthesis of nucleic acids.

It also supplies the precursors required for the synthesis of aromatic amino acids and vitamins. The pentose phosphate pathway does not directly produce energy; however, the NADPH generated serves as a potential source of ATP when its electrons are transferred to oxygen through the respiratory chain. NADPH is also extensively utilized in lipid metabolism.

This pathway occurs alongside glycolysis, in varying proportions, in many microorganisms. It is used, at least partially, by yeasts, molds, and numerous aerobic or facultatively anaerobic bacteria such as *Escherichia coli*. It plays an essential role in aerobic bacteria lacking the glycolytic pathway, including *Pseudomonas, Xanthomonas*, and *Acetobacter xylinum* (Fuentes-Lemus et al., 2025).

## I. Oxydative branche



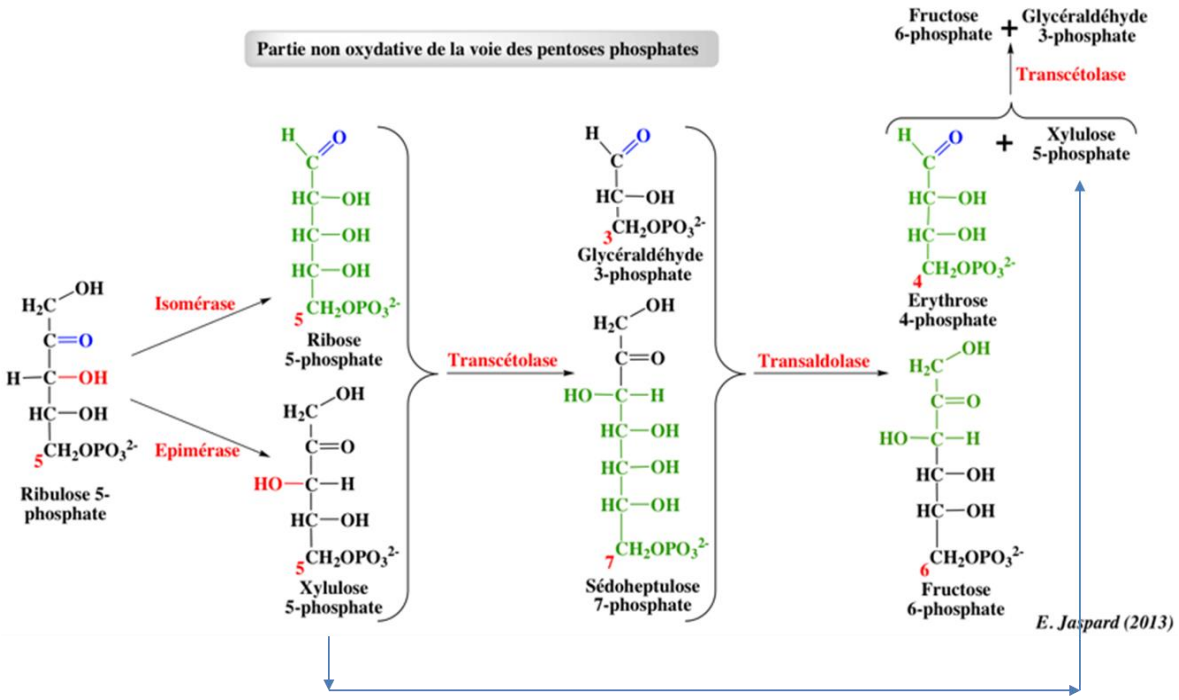

It consists of reactions 1 to 3 (shown in black), during which a molecule of glucose-6-phosphate is oxidized, resulting in the release of carbon dioxide ($CO_2$), a pentose phosphate, and two molecules of $NADPH,H^+$.

(1) Glucose-6-phosphate dehydrogenase

(2) Phosphogluconolactonase

(3) 6-Phosphogluconate dehydrogenase

## II. No oxydative branche

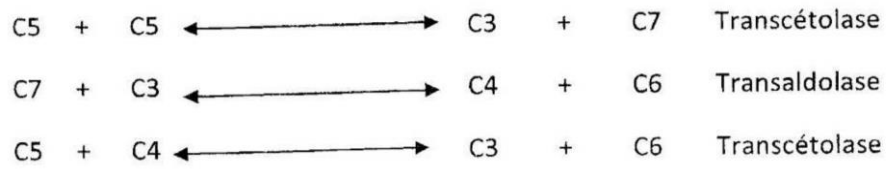


The **epimerase** interconverts ribulose-5-phosphate and xylulose-5-phosphate.

The **isomerase** converts ribulose-5-phosphate (a ketose) into ribose-5-phosphate (an aldose).

The **transketolase 1** transfers a two-carbon unit to either ribose-5-phosphate or erythrose-4-phosphate. This enzyme plays a crucial role in the *non-oxidative phase* of the pentose phosphate pathway. It catalyzes the transfer of a two-carbon unit (a glycolaldehyde group) from a ketose donor (such as xylulose-5-phosphate) to an aldose acceptor (such as ribose-5-phosphate or erythrose-4-phosphate). This reaction generates new sugar phosphates of different carbon lengths, such as sedoheptulose-7-phosphate (C7) and glyceraldehyde-3-phosphate (C3). The enzyme requires thiamine pyrophosphate (TPP) as a coenzyme, which acts as an electron sink to stabilize reaction intermediates (Horecker, 2002).

The **transaldolase** is an enzyme in the *non-oxidative phase* of the pentose phosphate pathway. It catalyzes the transfer of a three-carbon unit (dihydroxyacetone group) from a ketose donor (such as sedoheptulose-7-phosphate) to an aldose acceptor (such as glyceraldehyde-3-phosphate). This reaction produces fructose-6-phosphate (C6) and erythrose-4-phosphate (C4), which are important intermediates that can feed into glycolysis or be used for the synthesis of other biomolecules (Horecker, 2002).




This pathway operates parallel to glycolysis and mainly serves anabolic functions. It generates NADPH for reductive biosynthetic reactions (e.g., fatty acid and nucleotide synthesis) and produces pentose phosphates (such as ribose-5-phosphate) required for nucleic acid synthesis. It also allows the interconversion of sugars of different chain lengths, linking catabolism and anabolism (Horecker, 2002).

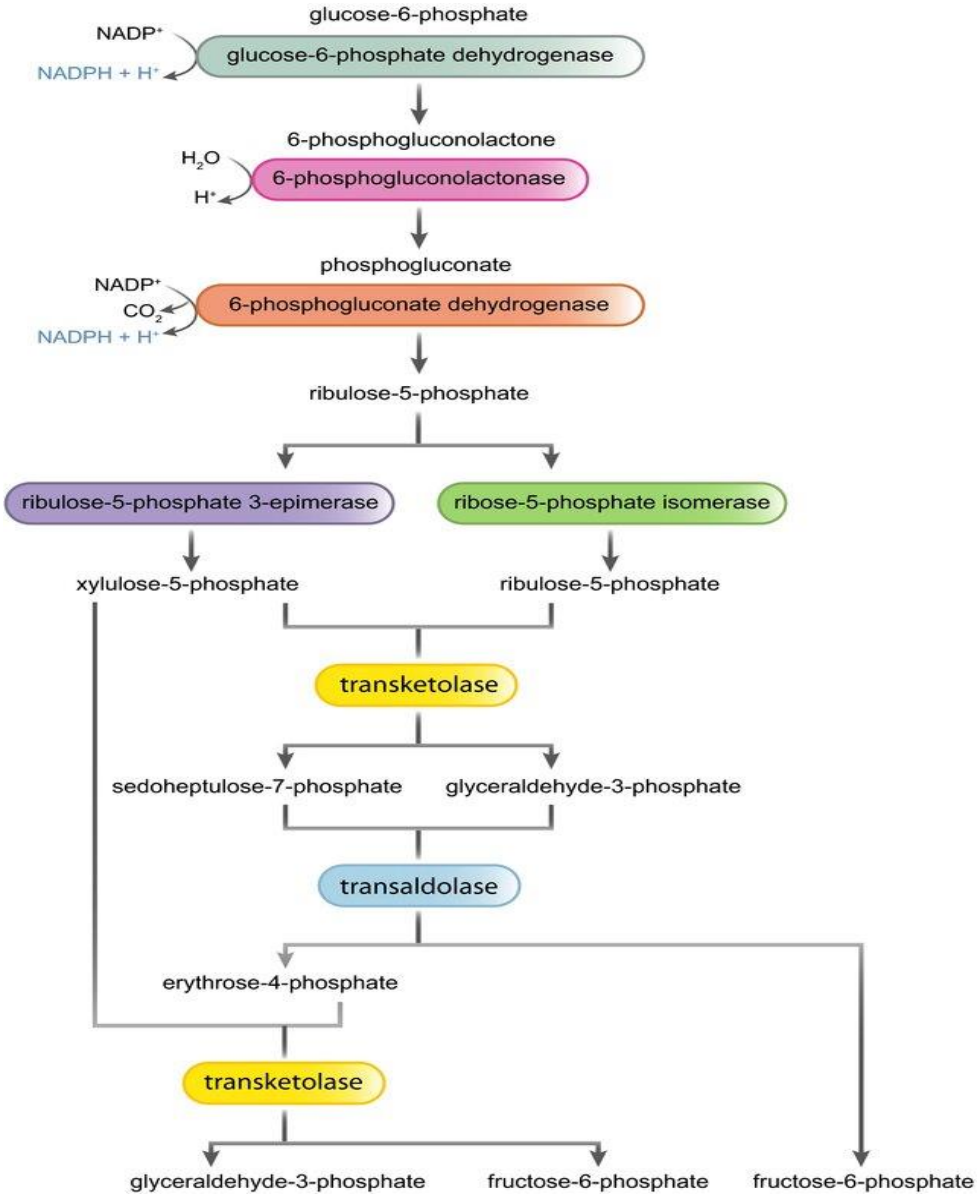



**Figure 13. Pentose phosphate pathway (McGuire, 2020)**

**c. Phosphoketolase pathway** (heterolactic fermentation)

This route is used by some lactic acid bacteria such as *Leuconostoc* and heterofermentative *Lactobacillus* species. It involves the cleavage of xylulose-5-phosphate by phosphoketolase, yielding glyceraldehyde-3-phosphate and acetyl-phosphate. This pathway produces lactic acid, ethanol (or acetate), $CO_2$, and one ATP per molecule of glucose.

**4-4- Fate of pyruvate**

Pyruvate is a central metabolic intermediate, produced at the end of glycolysis, and can follow multiple pathways depending on the organism, oxygen availability, and metabolic needs (Prochownik and Wang, 2021).

**a. Aerobic respiration**

Pyruvate is transported into the mitochondria (or cytoplasm in prokaryotes).

It is converted to acetyl-CoA by pyruvate dehydrogenase, releasing $CO_2$.

Acetyl-CoA then enters the Krebs cycle for complete oxidation to $CO_2$, generating NADH, $FADH_2$, and ATP via the electron transport chain (Prochownik and Wang, 2021).

✓ **Glyoxylate shunt**

The glyoxylate cycle is a metabolic pathway derived from the Krebs cycle that participates in anabolism in plants, bacteria, and fungi. It converts acetyl-CoA into succinate.

This cycle enables cells to utilize simple carbon compounds as carbon sources when more complex organic compounds, such as glucose, are unavailable.

Several microorganisms, including *Escherichia coli*, and many species of fungi can grow using acetate as their sole carbon and energy source. These organisms possess all the enzymes of the Krebs cycle, but additionally express two key enzymes specific to the glyoxylate cycle:

- Malate synthase (MS)
- Isocitrate lyase (ICL)

These enzymes allow the bypass of the decarboxylation steps of the Krebs cycle, enabling net synthesis of four-carbon intermediates from two-carbon units, which is essential for biosynthesis under carbon-limited conditions (Prochownik and Wang, 2021).

**Key enzymes and metabolites in the glyoxylate cycle**

Three enzymes of the glyoxylate cycle, malate dehydrogenase (MDH), citrate synthase (CS), and aconitase (ACO), are shared with the Krebs cycle. In addition, both cycles share several metabolites: malate, oxaloacetate, citrate, and isocitrate (Long et al., 2023).

The divergence between the two cycles occurs at isocitrate:

- Isocitrate lyase cleaves isocitrate into glyoxylate and succinate.

These steps allow simple carbon compounds to be subsequently used in the biosynthesis of biomolecules.

The cycle then continues as glyoxylate condenses with acetyl-CoA via malate synthase to form malate, which re-enters the Krebs cycle.

During the growth of microorganisms capable of utilizing acetate, oxaloacetate is decarboxylated to produce phosphoenolpyruvate (PEP), a key intermediate in the biosynthesis of hexoses and pentoses (Long et al., 2023).

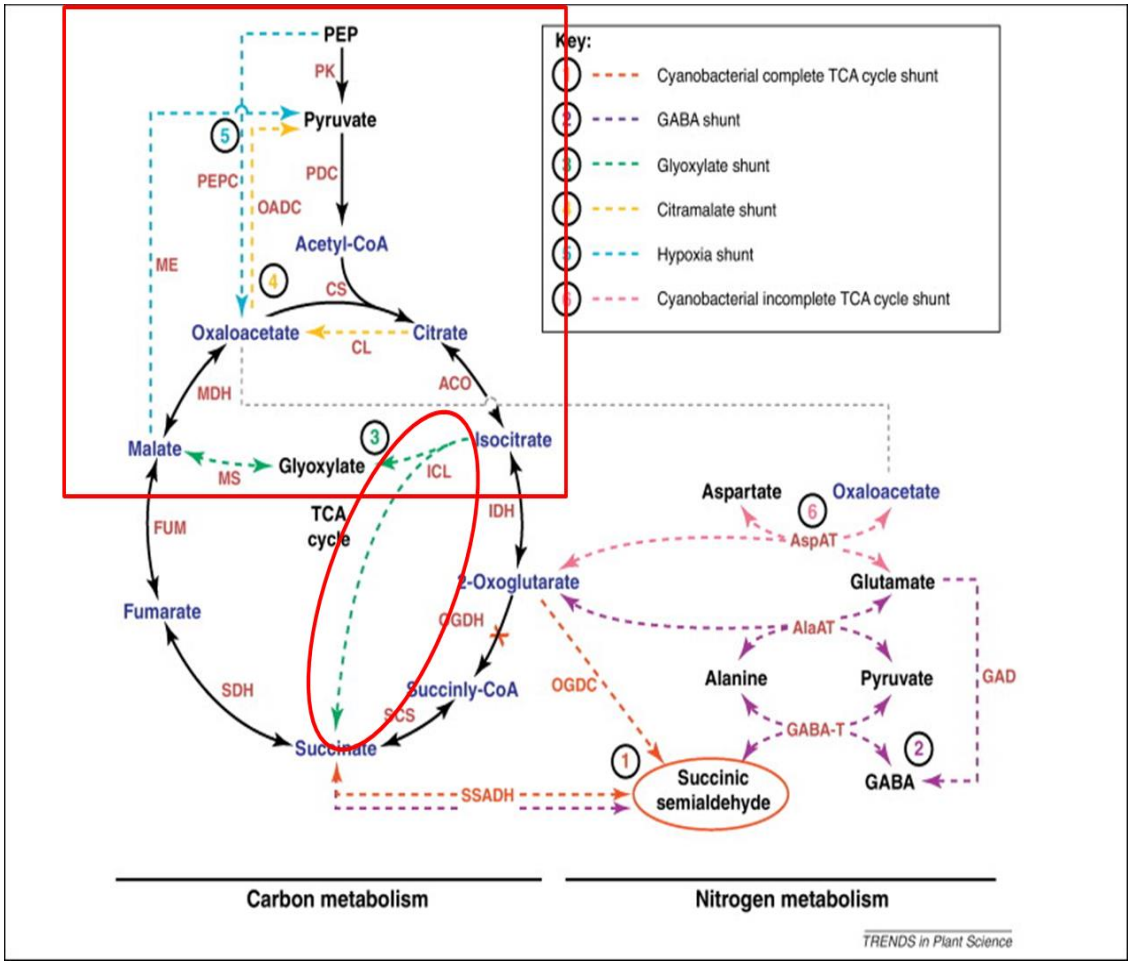



**Figure 14. Glyoxylate shunt representation (Long et al., 2023)**

**b. Anaerobic fermentation (absence of oxygen):**

Pyruvate serves as an electron acceptor to regenerate $NAD^+$ from NADH.

Depending on the organism:

- Lactic acid fermentation: Pyruvate → Lactate (catalyzed by lactate dehydrogenase)
- Alcoholic fermentation (yeasts): Pyruvate → Acetaldehyde + $CO_2$ → Ethanol

- Mixed acid or butanediol fermentation (bacteria): Pyruvate → Acetate, Lactate, Ethanol, 2,3-Butanediol, Formate, $H_2$, $CO_2$

2. **Anabolic pathways**

Pyruvate can serve as a precursor for amino acid synthesis (alanine, valine, leucine, etc.).

It can contribute to gluconeogenesis, regenerating glucose or other sugars.

In some microorganisms, pyruvate can lead to the formation of fatty acids or other biosynthetic intermediates.

**4-5- Study and significance of selected metabolic types**

**a. Aerobic lithotrophs**

Aerobic lithotrophs are microorganisms that obtain energy by oxidizing inorganic compounds rather than organic substrates. A classic example is nitrifying bacteria, which play a crucial role in the nitrogen cycle by converting ammonia ($NH_3$) to nitrite ($NO_2^-$) and subsequently to nitrate ($NO_3^-$).

These organisms are important in both natural ecosystems and industrial applications, such as wastewater treatment, because they contribute to nitrogen removal and recycling. Their metabolism is characterized by the coupling of electron transfer from inorganic donors to oxygen as the terminal electron acceptor, resulting in ATP synthesis via chemiosmosis (Cockell, 2011).

**b. Anaerobic lithotrophs**

Anaerobic lithotrophs are microorganisms that derive energy by oxidizing inorganic compounds in the absence of oxygen. Unlike aerobic lithotrophs, these organisms use alternative electron acceptors such as sulfates, carbon dioxide, or nitrates.

Sulfate-reducing bacteria (SRB): These bacteria oxidize inorganic substrates (e.g., hydrogen or organic compounds) and reduce sulfate ($SO_4^{2-}$) to hydrogen sulfide ($H_2S$). They play a critical role in anaerobic environments like sediments, wastewater systems, and the gut microbiota, contributing to the sulfur cycle.

Methanogenic bacteria: These archaea produce methane ($CH_4$) by reducing carbon dioxide ($CO_2$) with hydrogen ($H_2$) or by metabolizing other simple substrates such as acetate.

Methanogens are vital in anaerobic digestion and natural ecosystems like wetlands, where they help recycle carbon and generate bioenergy.

In both cases, energy conservation is achieved via electron transfer chains coupled to proton gradients or sodium ion gradients, driving ATP synthesis even in the absence of molecular oxygen (Cockell, 2011).

**c. Aerobic and anaerobic organotrophs**

Organotrophs are microorganisms that obtain energy by oxidizing organic compounds, such as carbohydrates, lipids, or proteins. They can be either aerobic or anaerobic, depending on whether oxygen serves as the terminal electron acceptor.

- Aerobic organotrophs: Species like *Pseudomonas* and acetic acid bacteria oxidize organic substrates in the presence of oxygen. This oxidation is coupled to electron transport chains, generating a proton gradient across the membrane and producing ATP through oxidative phosphorylation. Aerobic organotrophs often fully degrade substrates to $CO_2$ and $H_2O$, yielding a high energy efficiency.
- Anaerobic organotrophs: These microorganisms can oxidize organic compounds in the absence of oxygen by using alternative electron acceptors such as nitrates, sulfates, or organic compounds. The energy yield is generally lower than in aerobic respiration, and some anaerobic organotrophs perform fermentative metabolism, producing end products like lactate, ethanol, or organic acids.

Organotrophs play a crucial role in nutrient recycling, organic matter degradation, and industrial applications such as vinegar production, bioremediation, and fermentation-based food processes (Gerardi, 2003).