## 2. Metabolism

Microorganisms are capable of carrying out a wide variety of biochemical reactions, resulting in the production of biomass (cell bodies) and the degradation, transformation, or production of organic or mineral substances. For their survival (maintenance), their development (growth and multiplication), and the expression of their properties (motility, luminescence, etc.), microorganisms require energy and nutrients. Catabolism encompasses all the reactions that allow the recovery of biologically usable energy and the production of basic metabolites from organic substrates or cellular reserves. This degradation is more or less complete and results in the formation of metabolites (catabolic waste products). Anabolism encompasses all cellular synthesis reactions using basic metabolites derived from catabolism and elements from the environment.

### 2.1. Energy metabolism

#### 2.1.1. Energy sources and trophic types

The trophic type defines how a living organism builds its own organic matter and produces the energy it needs. Trophic types are analyzed along three axes:

- The nature of the carbon source
- The nature of the electron source
- The nature of the energy source

##### 2.1.1.1. Energy source

The energy required by microorganisms is supplied by light (phototrophic organisms) or by the oxidation of chemical substances (chemotrophic organisms). In both cases, the energy released in chemical form is stored in the cell as a high-energy compound, ATP (adenosine triphosphate). ATP is involved in all of the cell's energy processes. Synthetic reactions use the energy released by the breakdown of ATP into ADP:

$$ATP + H_2O \longrightarrow ADP + Pi + \text{énergie} \ (\Delta G^0 = -7.3 \text{ Kcal/mole}).$$

#### 2.1.1.1.1. Phototrophic organisms

Plants derive their energy from light, which is also essential for green algae, cyanobacteria, and some bacterial species. The photosynthesis process comprises two phases: the light phase and the dark phase. The light phase, or photophosphorylation, results in the formation of ATP, a reaction that generates energy usable by the cell.

#### 2.1.1.1.2. Chemotrophic organisms

Yeasts, molds, and most bacteria are incapable of photosynthesis. These microorganisms use the energy released during chemical oxidation reactions; they are called chemotrophs. Oxidation reactions occur in several ways. Some microorganisms (chemolithotrophs) derive their energy from the oxidation of mineral substances, while others derive it from organic substances (chemoorganotrophs). In most cases, the loss of electrons is coupled with a loss of protons. These electrons and protons reduce a final acceptor via a redox chain. ATP formation occurs largely during this electron and proton transport.

### 2.1.1.2. Electron source

Depending on the nature of the electron source taken from the environment by the bacterium, two different types are described: Organotrophy, the electron source is organic, example: glucose, fatty acid, and lithography, the electron source is mineral, example: ammonia, sulfur.

### 2.1.1.3. Carbon source

Depending on the organic nature of the carbon source taken up from the environment by the bacteria, two different nutritional types are described: carbon autotrophy, where the bacteria's carbon source is inorganic, such as carbon dioxide ($CO_2$) and monohydrogen carbonates ($HCO_3^-$) from the environment, and carbon heterotrophy, where the carbon source is organic, such as an organic molecule. These three fundamental axes, combined, underlie eight mechanisms used by microorganisms.

**Table I:** Eight trophic types in microorganisms

| Energy source | Source of electrons | Carbon source | Trophic type |
|---|---|---|---|
| **Light** **-Photo-** | Organic compounds -Organo- | Organic (e.g., glucose) -Heterotrophic | Photo-organo-heterotrophic |
| | | Mineral ($CO_2$) -Autotrophy | Photo-organo-autotrophy |
| | Mineral compounds -Litho- | Organic: -Heterotrophic | Photolitho-heterotroph |
| | | Mineral: -Autotrophy | Photo-litho-autotrophy |
| **Chemical Compounds (organic or Mineral) -Chemo-** | Organic compounds -Organo- | Organic: -Heterotrophic | Chemo-organo-heterotrophic |
| | | Mineral: -Autotrophy | Chemo-organo-autotroph |
| | Compounds (mineral) -Litho- | Organic: -Heterotrophic | Chemo-litho-heterotrophic |
| | | Mineral: -Autotrophy | Chemo-litho-autotroph |

### 2.1.2. Classification with respect to the final electron acceptor

#### 2.1.2.1. Aerobic respiration

Traditionally, when the final electron acceptor is molecular oxygen, it is called respiration, and microorganisms of this type are called aerobes. There are various mechanisms of aerobic respiration, but they can only occur under aerobic conditions. Microorganisms possessing only one such system are called "Obligate aerobes." Microorganisms that perform respiration possess an electron transport chain, the "respiratory chain" or "oxidative phosphorylation chain," linked to a cell membrane.

Oxidative phosphorylation is the process by which ATP is synthesized from the energy released during electron transfer. The most widely accepted hypothesis for ATP production is the chemiosmotic theory (or chemiosmotic coupling), formulated by the British biochemist Peter Mitchell in 1961. According to this hypothesis, in bacteria, the electron carriers of the respiratory chain are located in the cytoplasmic membrane, and there are many variants of this type. In contrast, in eukaryotes, they are located in the inner mitochondrial membrane. See **Figure 1**.

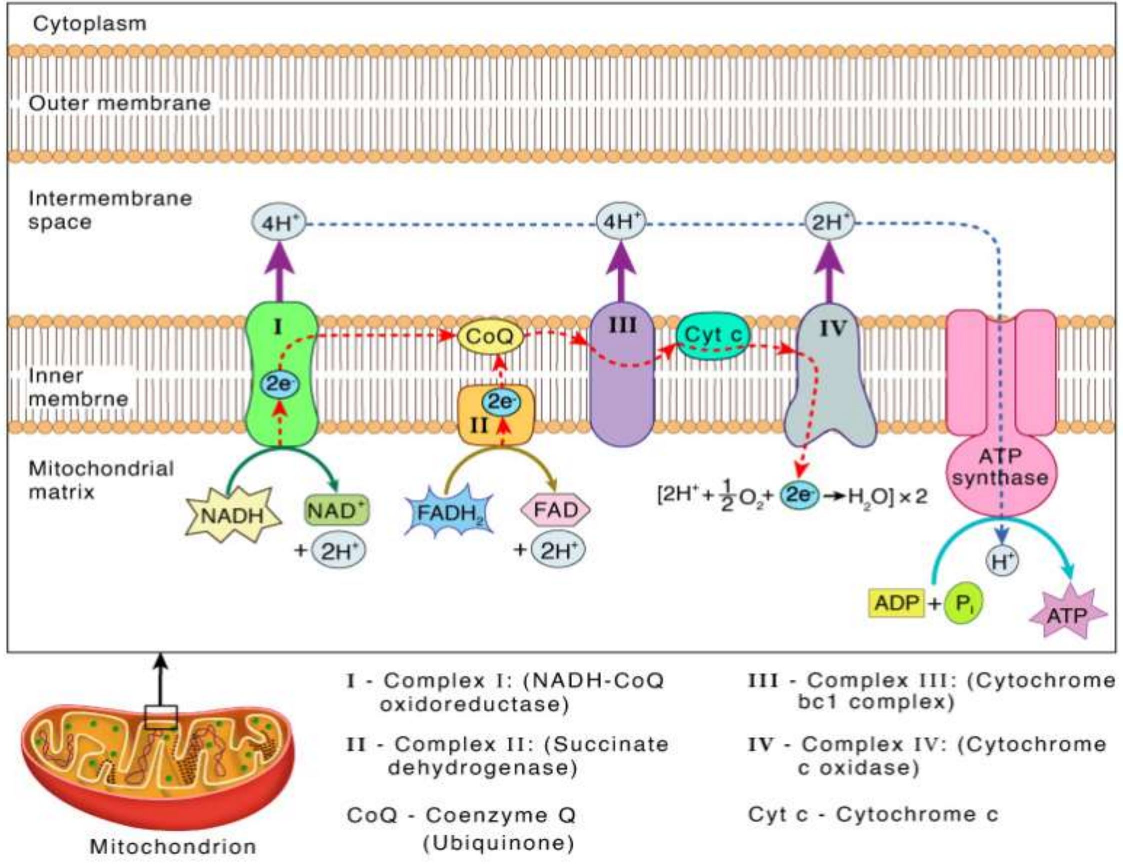


**Figure 1:** Mechanism of aerobic respiration.

In this scheme, the transporters are arranged asymmetrically in the inner membrane of eukaryotes or in the plasma membrane of bacteria, so that protons are transported across while electrons move along the chain. The release of protons into the intermembrane space in eukaryotes or the extracellular environment in bacteria occurs when electrons are transferred from one membrane electron transporter to another. Coenzyme Q transports electrons from complexes I and II to complex III. Cytochrome C moves electrons between complexes III and IV. Complex IV pumps electrons across the membrane, as they pass from cytochrome to oxygen, which is ultimately reduced by two electrons to form a water molecule. The movement

of electrons along the respiratory chain generates a concentration gradient of protons (electrochemical gradient or proton-motive force). The passage of protons across the membrane generates ATP. ATP synthesis occurs at the level of an enzyme located on the inner face of the cytoplasmic membrane; in bacteria, this enzyme is ATP synthase.

- **Oxidase test**

Place an oxidase disc impregnated with N-dimethylparaben on filter paper. Moisten the disc with distilled water using a platinum loop.  Take the bacterium to be identified (culture for 18 to 24 hours) and place it on this disc. The appearance of a violet color immediately indicates that the strain is oxidase-positive.

The presence of complex IV (aerobic respiration), the cytochrome oxidase enzyme, is of great importance for the identification of strictly aerobic bacteria. This is known as the oxidase test.

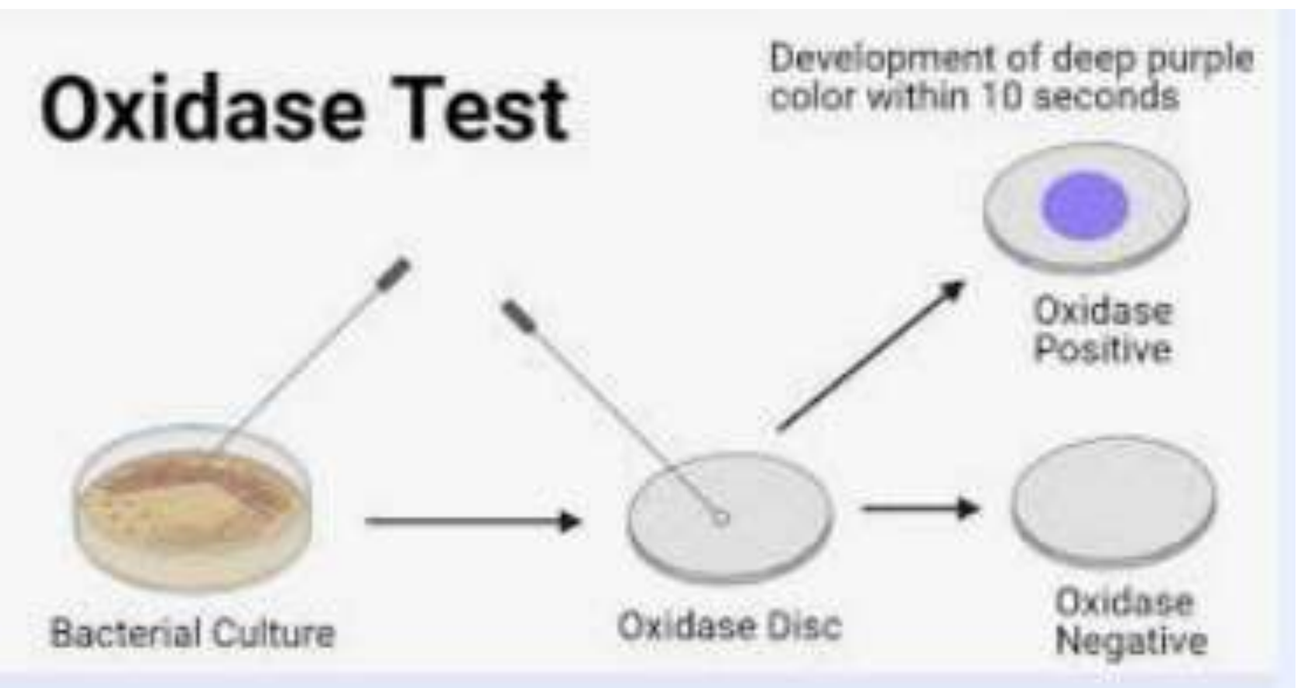


**Figure 2 :** Oxidase test.

### 2.1.2.2.Anaerobic respiration

This is a process where the final hydrogen acceptor is an oxidized mineral substance. Many microorganisms are capable of completely oxidizing glucose in the absence of air, provided that nitrate is present in the medium. Besides nitrates, other products can be used: sulfates, fumarate, $CO_2$, etc.

**Table II.** Different electron acceptors used during anaerobic respiration in bacteria.

| Accepteur d'électrons | Produit final réduit | Nom du processus | Exemples de microorganismes |
|---|---|---|---|
| $NO_3^-$ | $NO_2^-$, $NH_3$ or $N_2$ | Respiration anaérobie (dénitrification) | *Bacillus, Pseudomonas* |
| $SO_4^{--}$ | S or $H_2S$ | Respiration anaérobie (réduction des sulfates) | *Desulfovibrio* |
| fumarate | Succinate | Respiration anaérobie utilisant un accepteur d'e⁻ organique | *Escherichia coli* |
| $CO_2$ | $CH_4$ | Méthanogenèse | *Methanococcus* |

***Nitrate respiration***

Microorganisms that use nitrate as an electron acceptor reduce nitrate to nitrites by the action of nitrate reductase according to the following reaction:

$$NO_3^- + NADH, H^+ \longrightarrow NO_2^- + NAD^+ + H_2O$$

**Nitrate reductase test**

A few drops of Griess reagent are added to a culture in nitrate broth incubated for 24 to 48 hours at 37°C. After stirring, the reading is immediate.

**A-**Red coloration, *E-coli*: nitrates reduced to nitrites (nitrate reductase positive $NR^+$).

**B-**Once the medium remains colourless, add a little zinc powder (nitrate reducer) and shake well.

**C-**If the medium turns red, there are still nitrates, so these have not been reduced by the bacteria (nitrate reductase negative $NR^-$).

**D-**If the medium remains colorless, there are no more nitrates left; the bacteria have reduced them beyond the nitrite stage to nitrogen (nitrate reductase positive $NR^+$).

In some microorganisms (*Pseudomonas*), nitrates can be converted to nitrogen ($N_2$) (very active nitrate reductase). This process, called denitrification, is used by *Pseudomonas* and some species of *Bacillus* (denitrifying bacteria).

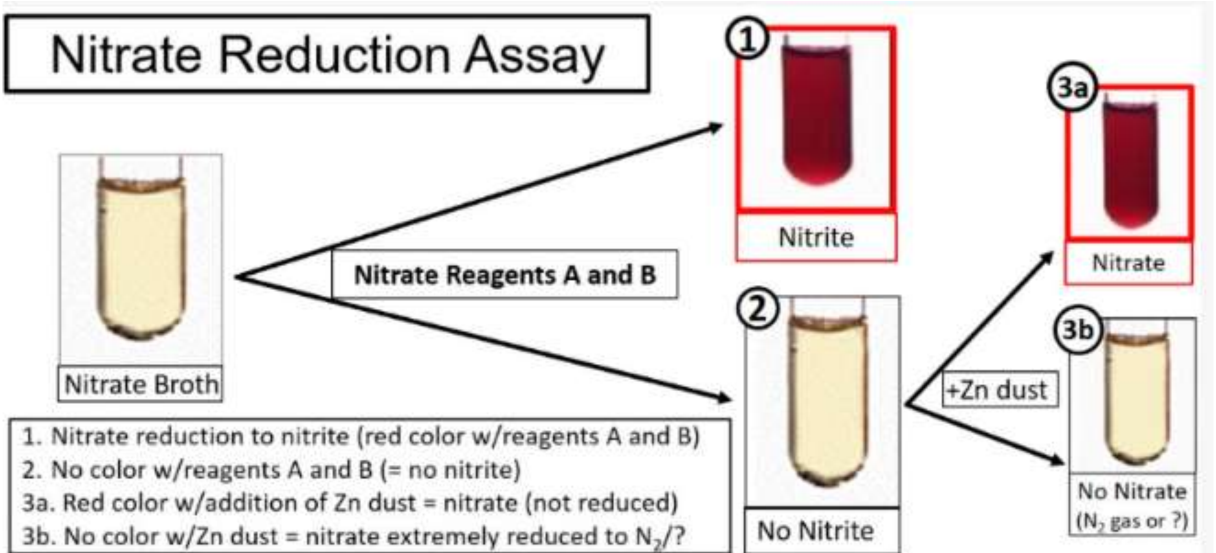


**Figure 3:** Nitrate reductase test**.**

**Sulfate reduction ($So_4^{2-}$) in sulfide (sulfide ion) $S_2^-$**

Sulfate reduction ($So_4^{2-}$) into sulfide (sulfide ion) $S_2^-$ in Desulfovibrio, then into hydrogen sulfide ($H_2S$) (hydrogen sulfide). *Desulfovibrio desulfuricans*: these are anaerobic bacteria with a generally respiratory metabolism using sulfate to replace oxygen in cellular respiration;therefore, these bacteria use sulfate or other sulfur compounds as final electron acceptors.

The reduction of $So_4^{2-}$ in $S_2^-$ then in $H_2S$ occurs according to the reaction:

$$So_4^{2-} + 8\ H+ + 8e \rightarrow S_2^- + 4\ H_20 \rightarrow H_2S$$

These sulfur-reducing bacteria are of great importance in the recycling of sulfur in ecosystems.

### 2.1.2.3.Fermentation

In fermentation, the final electron (and proton) acceptor is an organic molecule. Many fermentations can occur anaerobically because all the electrons and protons released from substrate oxidation are used to reduce the organic acceptor (for example, in homolactic fermentation). Fermentation is used by facultative anaerobic bacteria, which preferentially use respiration when possible, and strict anaerobic bacteria (*Clostridium*). The energy yield of fermentation is lower than that of respiration. NADH from glycolysis is oxidized when used to reduce pyruvate or a pyruvate derivative (X). The resulting product is lactate, which is a reduced product Y.

Aerobic respiration > anaerobic respiration > fermentation.

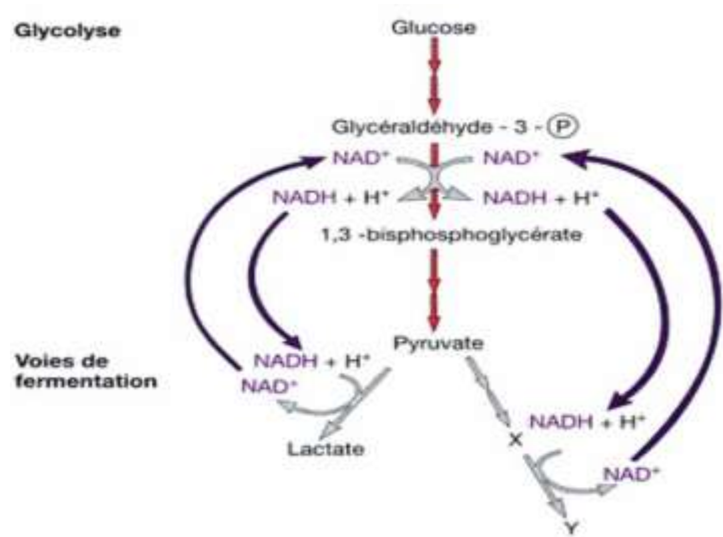


**Figure 4:** The oxidation of NADH during fermentation.

### 2.1.3. Classification based on their relationship with oxygen

There are several classes of bacteria depending on their relationship with $O_2$

- Strict aerobes only develop in the presence of air. Their main source of energy is respiration. Molecular oxygen, the ultimate electron acceptor, is reduced to water (*Pseudomonas*).

- Microaerophiles grow best or exclusively when the partial pressure of oxygen is lower than that of air (*Mycobacteriaceae*).
- Facultative anaerobes grow with or without air: enterobacteria (*Escherichia*, *Salmonella*). Energy comes from the oxidation of substrates and the fermentation pathway.
- Strict anaerobes only thrive in the complete or near-total absence of oxygen, which is most often toxic. This is the case for intestinal bacteria (*Clostridium*) and many bacteria present in the body's normal flora. For some anaerobic bacteria (and strict anaerobes), oxygen is unnecessary and often even toxic. Indeed, oxygen can, through chemical reactions, produce hydrogen peroxide ($H_2O_2$) which can oxidize lipids and proteins of the bacterial cell. These bacteria do not have protective enzymes such as catalase.

**Catalase test**

Place a drop of hydrogen peroxide in the center of the slide. Take the platinum loop and the loop wire, and carefully ignite it until both the loop and the wire glow red. Wait a few seconds, then remove it and allow it to cool in a sterile field. Take your petri dish containing the strain to be identified and scrape a few colonies with the platinum loop. Then, place the collected bacteria into the drop of hydrogen peroxide and observe for the formation of bubbles or effervescence. If bubbles form, the bacteria synthesize the catalase enzyme (catalase positive); if not, the bacteria do not synthesize it (catalase negative). Remember to ignite the platinum loop a second time, as some liquid remains (this is necessary to sterilize and burn off any remaining bacteria).

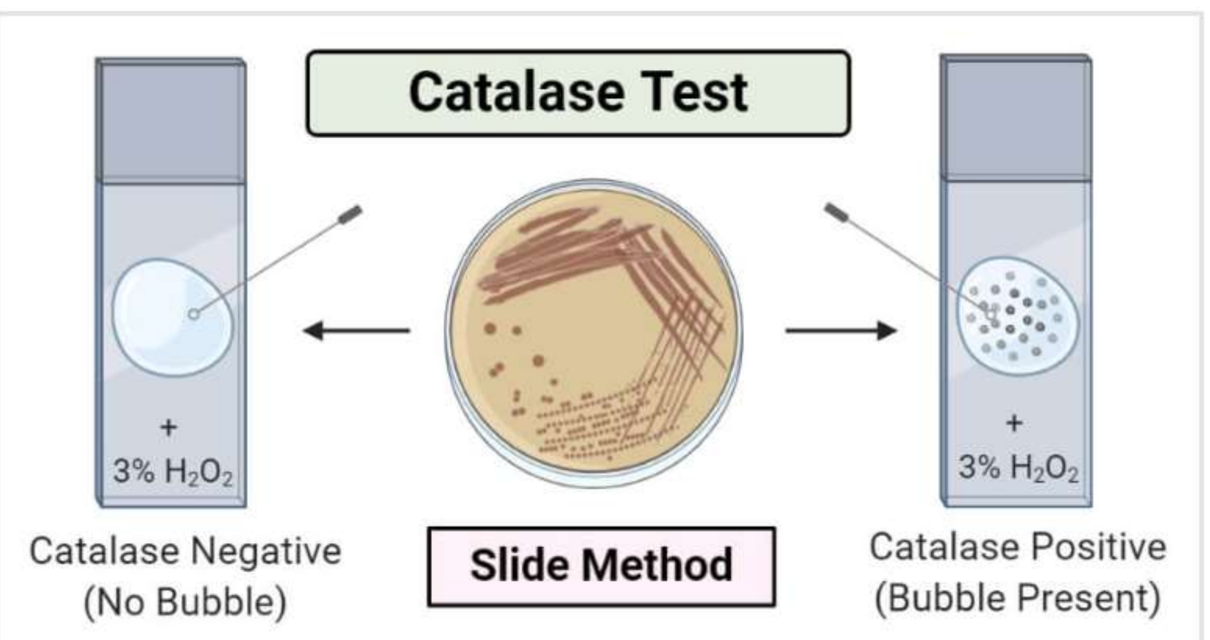


**Figure 5:** Catalase test

### 2.2. Carbohydrate catabolism

Carbohydrate metabolism is the set of various processes biochemical responsible for the formation, degradation and inter-conversion of carbohydrates in living organisms. Carbohydrate catabolism refers to the chemical reactions that lead to the breakdown of molecules (catabolism) of carbohydrates to produce energy.

The carbohydrates that can be broken down by microorganisms are numerous and varied. Polysaccharides such as starch, cellulose, inulin, and sometimes even smaller molecules like sucrose are unable to enter the cell. They must first be broken down into low molecular weight fragments by hydrolytic enzymes secreted by the microorganism into the surrounding environment. The resulting products then enter the cell. In most cases, the transformation of carbohydrate macromolecules, as well as various other organic substances, results in the formation of hexoses (primarily glucose) or pentoses. Glucose is the starting point for the main catabolic pathways.

#### 2.2.1. Starch degradation

Starch constitutes the main carbohydrate reserve in plants; it contains two polysaccharides in varying proportions: amylose (the major constituent) and amylopectin (the minor constituent). Amylose is a flexible molecule with a linear structure, consisting of several

hundred α-D-glucopyranose residues linked by 1-4 linkages. Amylopectin is also a glucose polymer, composed of linear chains similar to those of amylose, but linked together by α(1-6) linkages. The branch points are approximately 20 to 30 glucose units apart. Microbial amylases can be classified into two main groups based on their mode of attack:

- **α-amylase or α(1-4)-glucan glucanohydrolase**
- **Glucoamylase or α(1-4)-glucan glucohydrolase**

### 2.2.2. Cellulose degradation

Cellulose is a linear polymer of D-glucose, with glucose molecules linked together by β (1-4) bonds. Cellulolytic microorganisms are found in a wide variety of bacterial genera (*Bacillus*) and molds (*Aspergillus*, *Fusarium*), which play a key role in the carbon cycle. These enzymes are rare in yeasts.

### 2.2.3. Sucrose degradation

Sucrose is first hydrolyzed into glucose and fructose by invertase, present in many yeasts (*Saccharomyces cerevisiae*), many molds (*Aspergillus niger*), and many bacteria (*Streptococcus*). After lactose hydrolysis, the resulting glucose is broken down by one of the pathways described later.

### 2.2.4. Lactose degradation

Many microorganisms possess β-galactosidase: yeasts (*Candida*…), molds (Aspergillus…), bacteria (*E. coli*, *Lactobacillus, Bacillus*…). After lactose hydrolysis, the glucose formed is degraded by one of the pathways described later.

### 2.2.5. Maltose degradation

It is generally hydrolyzed into 2 glucose molecules by a maltase (or glucoamylase).

### 2.2.6. Glucose catabolism

The oldest known hexose degradation pathway is glycolysis, which leads to the transient formation of pyruvic acid. Alternatives to glycolysis exist in a wide variety of aerobic and anaerobic microorganisms. These pathways are used either exclusively or concurrently with glycolysis.

#### 2.2.6.1.Glycolysis or Embden-Meyerhof-Parnas (EMP) pathway

This pathway, known as the hexose diphosphate pathway, is a series of reactions that transform glucose into two pyruvate molecules, producing two NADH molecules and two ATP molecules (4 ATP molecules formed by phosphorylation at the substrate level and 2 ATP molecules consumed). Glycolysis is very widespread among microorganisms: yeasts, molds, and anaerobic bacteria (Enterobacteriaceae).

The key points in the glycolysis pathway are:

- Activation of glucose in the form of glucose-6P by means of ATP, isomerization and second phosphorylation to give fructose-1, 6-diphosphate and two ADPs.
- Cleavage of fructose-1, 6 diP into two triose-phosphate molecules, under the action of aldolase (enzyme characteristic of this metabolic pathway).

-Isomerization of 3-phosphoglyceraldehyde/dihydroxyacetone-phosphate and dehydrogenation with reduction of $NAD^+$. This reaction is accompanied by phosphorylation at the substrate level and leads to the formation of 1, 3 diphosphoglycerate (possesses a high-energy bond).

- Transfer of a phosphoric ester bond from 1, 3-diphosphoglycerate to ADP.
- Transfer of the phosphoric ester bond from phosphoenolpyruvate to ADP and formation of pyruvate and ATP.

The outcome of the Process is:

$$\textbf{Glucose} + \textbf{2NAD}^+ + \textbf{2 ADP} + \textbf{2Pi} \rightarrow \textbf{2 pyruvate} + \textbf{2NADH} + \textbf{2H}^+ + \textbf{2ATP} + \textbf{H}_2\textbf{O}$$

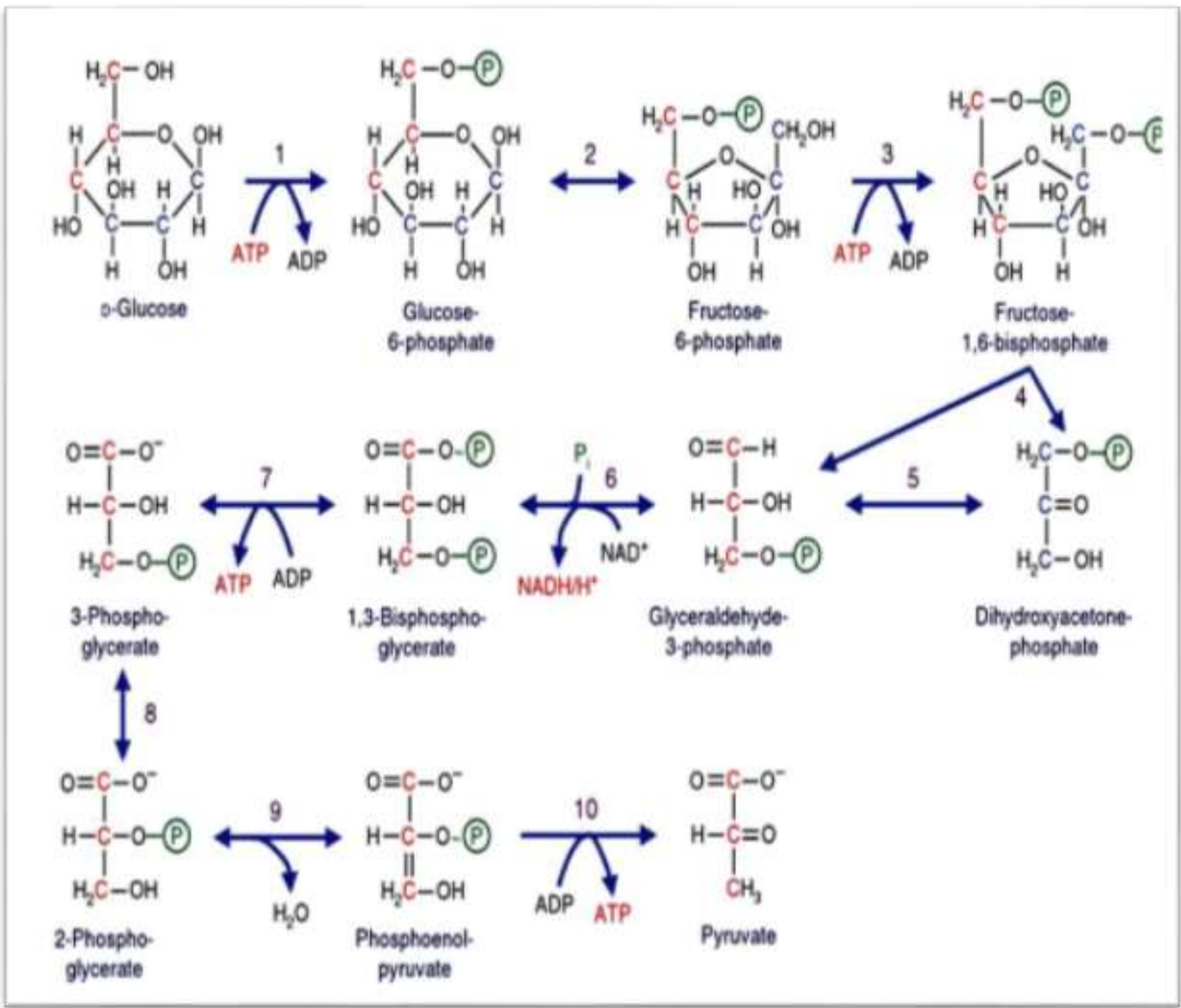


**Figure 6:** Glycolysis pathway (Embdem-Meyerhof-Parnas pathway).

(1) (ATP-dependent) hexokinase, (6) glyceraldehyde-3-phosphate dehydrogenase (GAPDH),
(2) phosphoglucoisomerase (PGI), (7) phosphoglycerokinase (PGK),
(3) (ATP-dependent) phosphofructokinase (PFK), (8) phosphoglycerate mutase (PGM),(4) fructose bisphosphate aldolase (FBA),(9) (phosphoglycerate) enolase,
(5) triose phosphate isomerase, (10) Pyruvate kinase (PYK)

### 2.2.2.6.2. Alternatives to glycolysis

#### 2.2.6.2.1. Pentose phosphates or hexose monophosphates

This aerobic pathway is very important because it provides pentoses, required for the synthesis of nucleic acids and prosthetic groups containing nucleotides. It also provides the building blocks necessary for the synthesis of aromatic amino acids and vitamins. The hexose monophosphate pathway does not directly produce energy, but the $NADPH_2$ formed is a source of ATP when electrons are transported to oxygen via the respiratory chain; $NADPH_2$ can also be used by lipid metabolism.

This pathway, alongside glycolysis in varying proportions, is present in many microorganisms. The initial steps lead to the formation of gluconate-6-phosphate and are common to other respiratory and fermentative pathways. From gluconate-6-phosphate, ribulose-5-phosphate is formed, the starting point of the pentose-phosphate oxidative cycle (Figure 7):

$$3\text{glucose-6p} + 6NADP^{+}\ 3H_2O - \rightarrow 2\ \text{fructose-6P} + \text{Glyceraldéhyde-3P} + 3CO_2 + 6NADPH, H^{+} + 6H^{+}$$

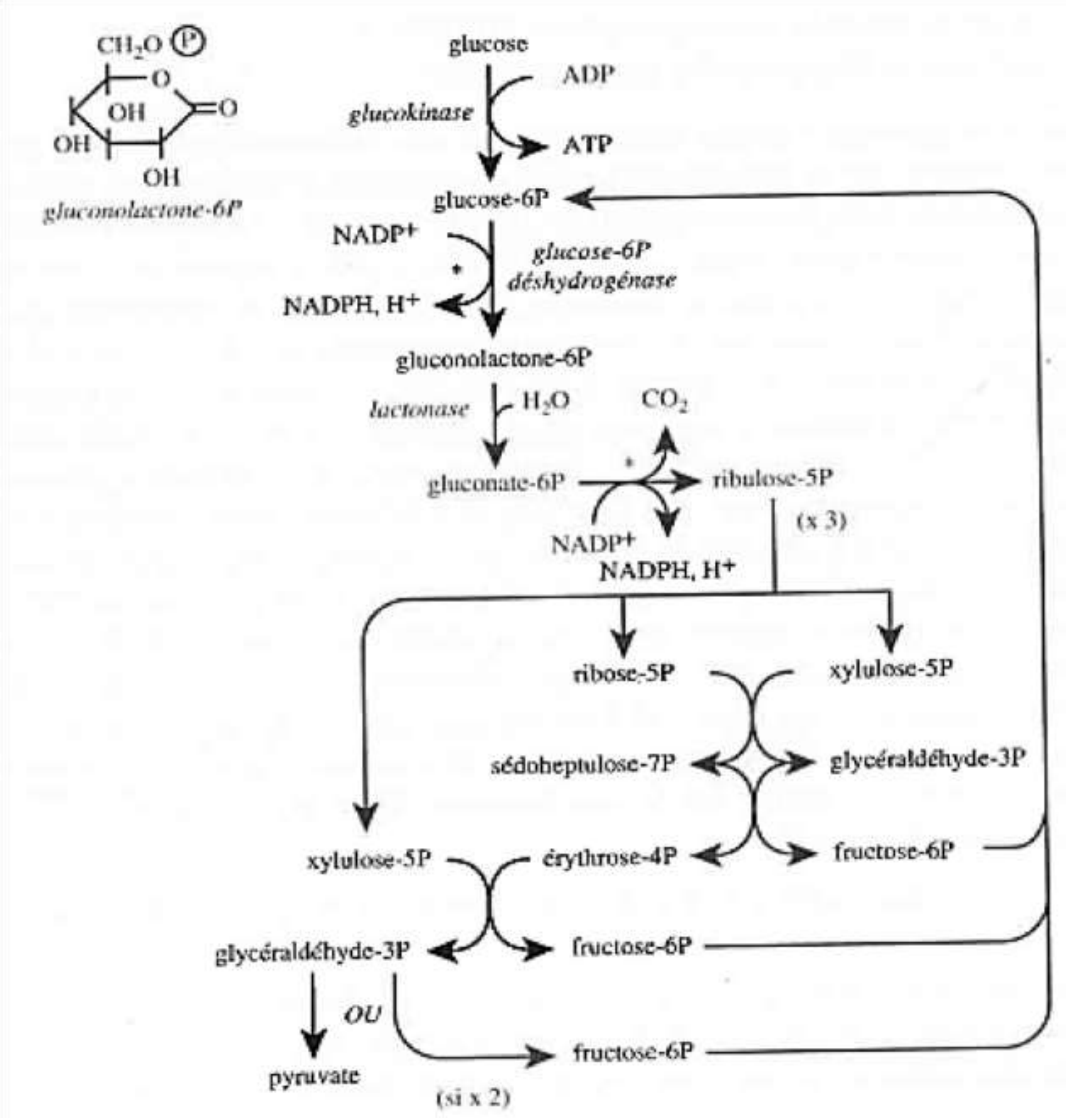


**Figure 7:** Hexose monophosphate pathway

The connections between the glycolysis pathway and the hexose monophosphate pathway are numerous (Figure 8). Glyceraldehyde-3-phosphate can be converted to pyruvate. Pyruvate is used by the pathways we will see later (pyruvate metabolism). Glyceraldehyde-3-phosphate can also be condensed to fructose-6-phosphate by glyceraldehyde-6-phosphate aldolase.

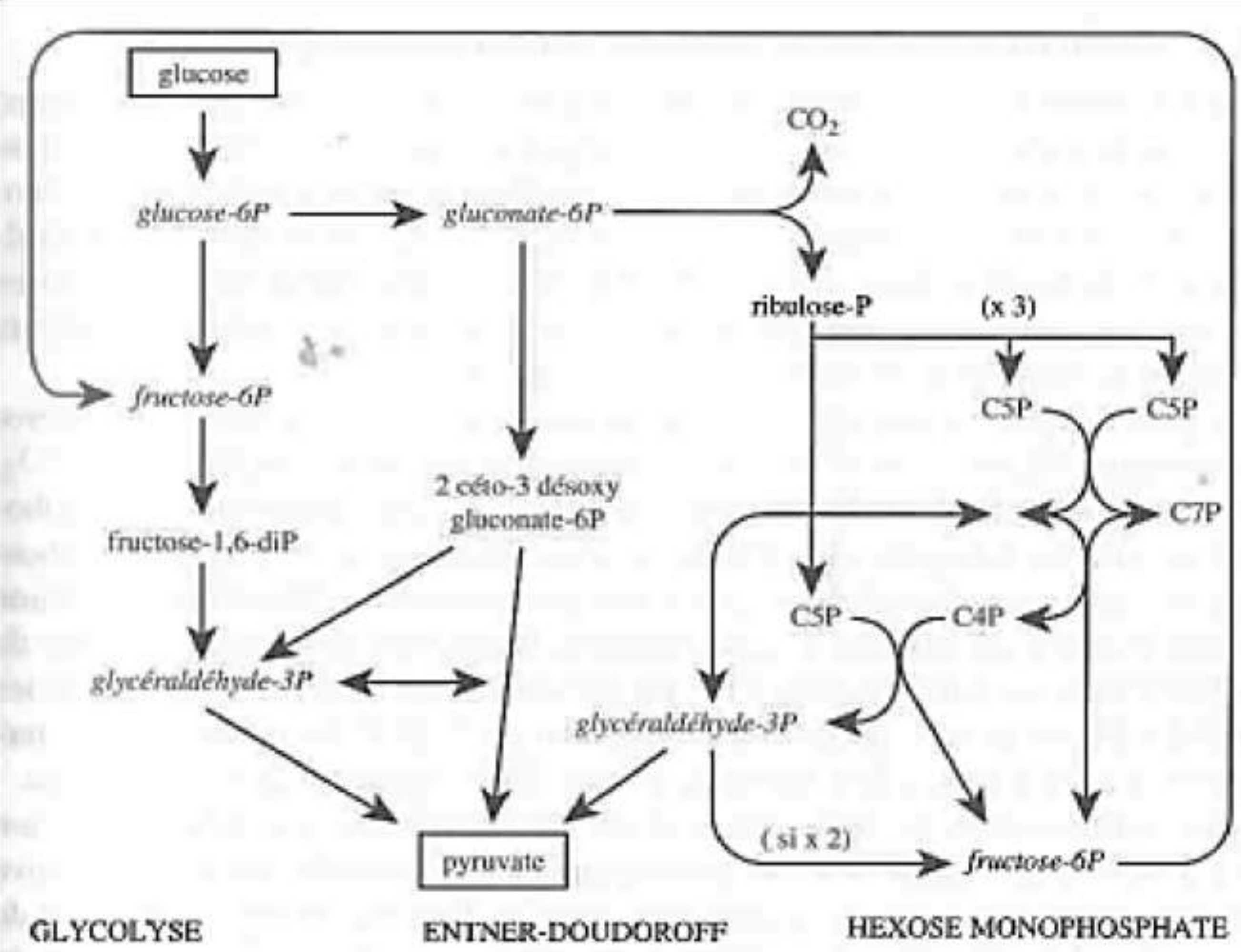


**Figure 8:** Glycolysis and other pathways

The pentose phosphate pathway can be broken down into 3 parts:

- An oxidative part: a series of reactions that oxidize glucose-6-phosphate, reduce $NADP^+$ to NADPH, and result in the formation of ribulose-5-phosphate; a non-oxidative part: reversible isomerization and epimerization reactions
- A non-oxidative part: transketolization and transaldolization reactions (transfer of groups containing several carbons).

❖ **Oxidative stages**

Glucose-6-phosphate dehydrogenase catalyzes the oxidation of the aldehyde (hemiacetal) group attached to carbon C1 of glucose-6-phosphate to form a carboxylic acid in an ester bond, a lactone. $NADP^+$ acts as an electron acceptor. This reaction is irreversible. 6-Phosphogluconolactonase catalyzes the hydrolysis of the lactone and opens the ring to form 6-phosphogluconate. Phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate to form ribulose-5-phosphate. The hydroxyl group at position C3 of 6-

phosphogluconate is oxidized to a ketone, which promotes the loss of the carboxyl group at C1 as $CO_2$. $NADP^+$ acts as an electron acceptor.

Ribulose 5-phosphate is also a key intermediate in the Calvin cycle (photosynthesis).

- **Non-oxidative (reversible) steps**
  - **Isomerization and epimerization step**

- Epimerase interconverts ribulose-5-phosphate and xylulose-5-phosphate
- Isomerase transforms ribulose-5-phosphate (ketose) into ribose-5-phosphate (aldose)

  - **Transketolization and transaldolization step**

- **Transketolization**

The transketolation reaction involves transferring a two-carbon group ($CH_2OH$-CO) from xylulose 5-phosphate to ribose 5-phosphate. The enzyme that catalyzes this reaction is transketolase. This process yields sedoheptulose 7-phosphate and 3-phosphate D-glyceraldehyde.

- **Transaldolization**

The reaction involves transferring a group of three carbon atoms from the $CH_2OH$-CO-$CH_2OH$ group of sedoheptulose 7-phosphate to 3-phosphate D-glyceraldehyde. The enzyme that catalyzes this reaction is transaldolase. This process yields erythrose phosphate and fructose 6-phosphate.

- **Transketolization**

Transketolase transfers the two-atom group from the carbon of xylulose 5-phosphate to erythrose 4-phosphate. This yields fructose 6-phosphate and 3-phospho D-glyceraldehyde.

This route:

- ✓ It is an alternative to glycolysis with a more anabolic (biosynthesis) than catabolic (degradation) purpose.
- ✓ It exists in all eukaryotes and almost all bacteria. It is independent of oxygen (it takes place in aerobic and anaerobic conditions).

- ✓ The production of a reducing power in the form of NADPH which is then used in particular for the biosynthesis of fatty acids.
- ✓ The production of pentoses, in particular ribose-5-phosphate used for the biosynthesis of pyridine coenzymes ($NAD^+$ and NADP+), flavin coenzymes (FMN and FAD), coenzyme A and for the biosynthesis of nucleotides.
- ✓ The production of erythrose-4-phosphate, a precursor of aromatic amino acids.
- ✓ Ribulose 5-phosphate is also a key intermediate in the Calvin cycle (photosynthesis).

**2.2.6.2.1. 2-Keto-3-deoxygluconate or Entner-Doudoroff pathway**

This pathway shares common steps with both the hexose monophosphate pathway and glycolysis. It was discovered by Entner and Doudoroff. The essential steps of this pathway are:

- Activation of glucose by ATP.
- Oxidation of the aldehyde group of glucose-6P to form 6-phosphogluconate with parallel reduction of $NADP^+$.
- Dehydration of 6-phosphogluconate and formation of CDPG or KDPG (2-keto-3-deoxy 6-phosphogluconate).
- Cleavage by CDPG-aldolase to give on the one hand glyceraldehyde-3P and on the other hand pyruvate.
- Transformation of glyceraldehyde-3-phosphate into pyruvate via glycolysis, with the formation of 2 moles of ATP and 1 mole of $NADH_2$ per mole of triose phosphate. For one molecule of glucose, 1 ATP, 1 $NADPH_2$, and 1 $NADH_2$ are formed.

**2.2.7. Aerobic metabolism of pyruvate**

In the presence of air, strict or facultative aerobic microorganisms ensure the complete oxidation of glucose. The pyruvate formed is oxidized by the Krebs cycle and the glyoxylate shunt.

**2.2.7.1. Krebs cycle (tricarboxylic acid cycle "TCA" or citric acid cycle)**

The Krebs cycle is the aerobic oxidation pathway of acetate produced not only by glycolysis or the hexose monophosphate shunt. The cycle provides the starting compounds for respiratory reactions. A maximum of 3 ATP molecules are formed per electron pair transported between NAD and oxygen. The overall yield per mole of glucose oxidized via glycolysis and

the Krebs cycle is therefore a maximum of 38 ATP. The Krebs cycle cannot function under anaerobic conditions because succinate dehydrogenase and α-ketoglutarate dehydrogenase are inactive.

(1) (ATP-dependent) hexokinase (aka, glucokinase), (2) glucose-6-phosphate dehydrogenase (Glc-6PDH), (3) 6-phosphogluconolactonase, (4) 6-phosphogluconate dehydratase, and (5) 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase.

**Figure 9**: Entner-Doudoroff pathway.

The first step in this process employs a multi-enzyme system, the pyruvate dehydrogenase complex (an association of three enzymes acting sequentially to catalyze the oxidative decarboxylation of pyruvate to acetyl-CoA). This complex oxidizes and cleaves pyruvate to form CO2 and acetyl-coenzyme A.

$$\text{Pyruvate} + \text{NAD+} + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{NADH, H}^+$$

Acetyl-CoA is energy-rich because a high-energy thiol (-SH) sulfhydryl group links acetic acid to coenzyme A. Acetyl-CoA then enters the tricarboxylic acid cycle (TCA cycle)

also called the citrate cycle or Krebs cycle.

✓ In the first reaction, acetyl CoA, under the action of the enzyme citrate synthase, condenses with oxaloacetate to form citrate, a six-carbon molecule.

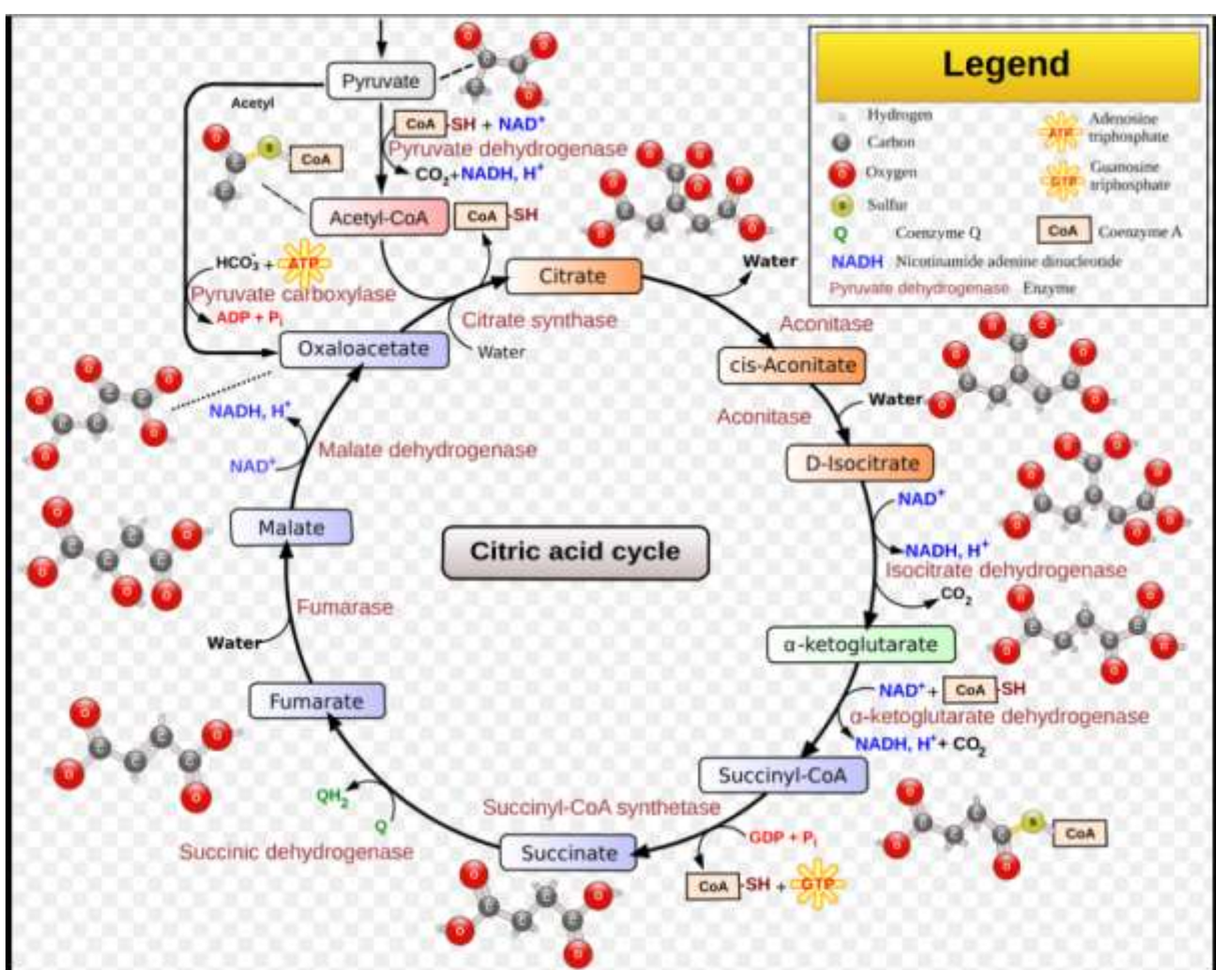


**Figure 10** : Krebs cycle.

- Citrate, in turn, is rearranged by the enzyme aconitase to give isocitrate, a more easily oxidized secondary alcohol. Isocitrate is subsequently oxidized and decarboxylated twice by the enzyme isocitrate dehydrogenase to give α-ketoglutrate (five carbons). This overall reaction occurs in two steps.
- Then, α-ketoglutarate is taken up by the α-ketoglutarate dehydrogenase enzyme complex to yield succinyl-CoA (four carbons), a molecule with a high-energy bond. At this stage, two NADH molecules have been formed and two carbons have exited the cycle as $CO_2$. The cycle continues with the conversion of succinyl-CoA to succinate by the enzyme succinyl-CoA ligase. The high-energy bond of succinyl-CoA is broken, and

the released energy is used to form GTP by substrate phosphorylation. GTP is also an high-energy molecule, functionally equivalent to ATP.

- Next, there is the dehydrogenation of succinate to fumarate by FAD-dependent succinate dehydrogenase. This catalysis generates a molecule of $FADH_2$.
- The penultimate reaction involves the hydration of fumarate to L-malate by fumarase. The final oxidation step regenerates oxaloacetate, and as long as it is supplied with acetyl CoA, the cycle repeats.
- The ATC cycle generates two $CO_2$, three NADH, one $FADH_2$, and one GTP for each molecule of acetyl-CoA oxidized. The enzymes of the cycle are widespread among microorganisms. In prokaryotes, they are located in the cytoplasm. In eukaryotes, they are found in the mitochondria.

#### 2.2.7.2.Glyoxylic shunt

A number of microorganisms are capable of growing using acetate as their sole source of carbon and energy. These organisms possess all the enzymes of the Krebs cycle, but also two additional enzymes:

- Isocitrate which cuts isocitrate into succinate and glyoxylate

- Malate synthetase, which condenses glyoxylate with acetyl CoA to form malate.

The glyoxylic shunt provides no biologically usable energy. It only functions when the microorganism is cultured on acetate because glucose represses the previously mentioned enzymes. During growth on acetate, the cells decarboxylate oxaloacetate to provide phosphoenolpyruvate, the starting point for the biosynthesis of hexoses and pentoses.

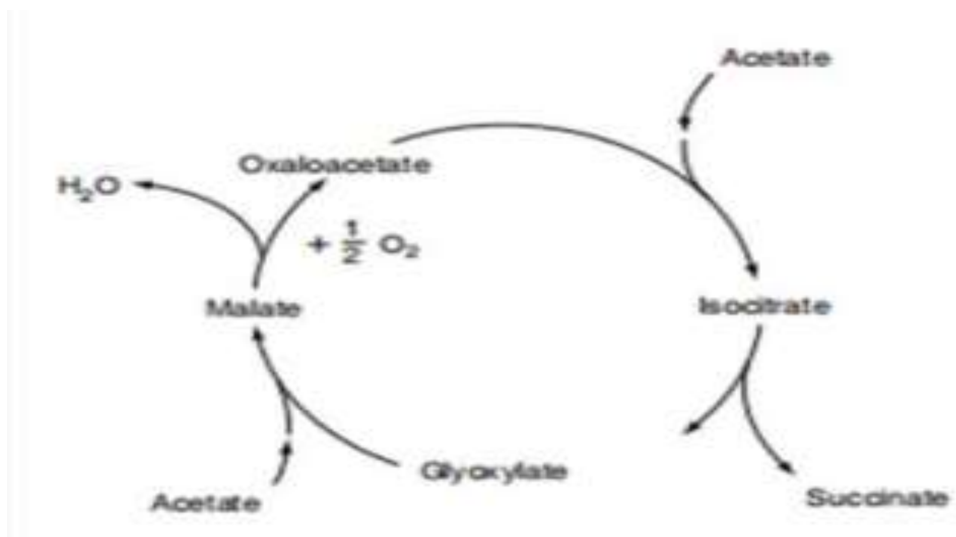


**Figure 11**. Glyoxylic cycle

### 2.2.8. Lipid catabolism

Microorganisms often use lipids as an energy source. Triglycerides or triacylglycerols, glycerol esters, and fatty acids are common energy sources. Triglycerides are hydrolyzed into fatty acids and glycerol by lipases or less specific esterases, often exocellular. These lipases are found in molds (*Aspergillus…*), yeasts (*Candida*) and bacteria (*Pseudomonas*).

Glycerol is phosphorylated and oxidized to dihydroxyacetone-P and degraded in glycolysis. Fatty acids, on the other hand, are catabolized by a process (cycle) called β-oxidation. ATP first activates them in the presence of coenzyme A to form acyl-CoA, which is then oxidized to β-ketoacyl-CoA. After hydrolysis, acetyl-CoA and an acyl-CoA with two fewer carbons are formed. The oxidation reactions continue as long as necessary, depending on the length of the carbon chain. The acetyl-CoA formed can be incorporated into the Krebs cycle and the glyoxylic shunt. The NADH and $FADH_2$ produced, respectively, can be oxidized by the electron transport chain to produce ATP. Fatty acids constitute a rich energy source for microbial growth.

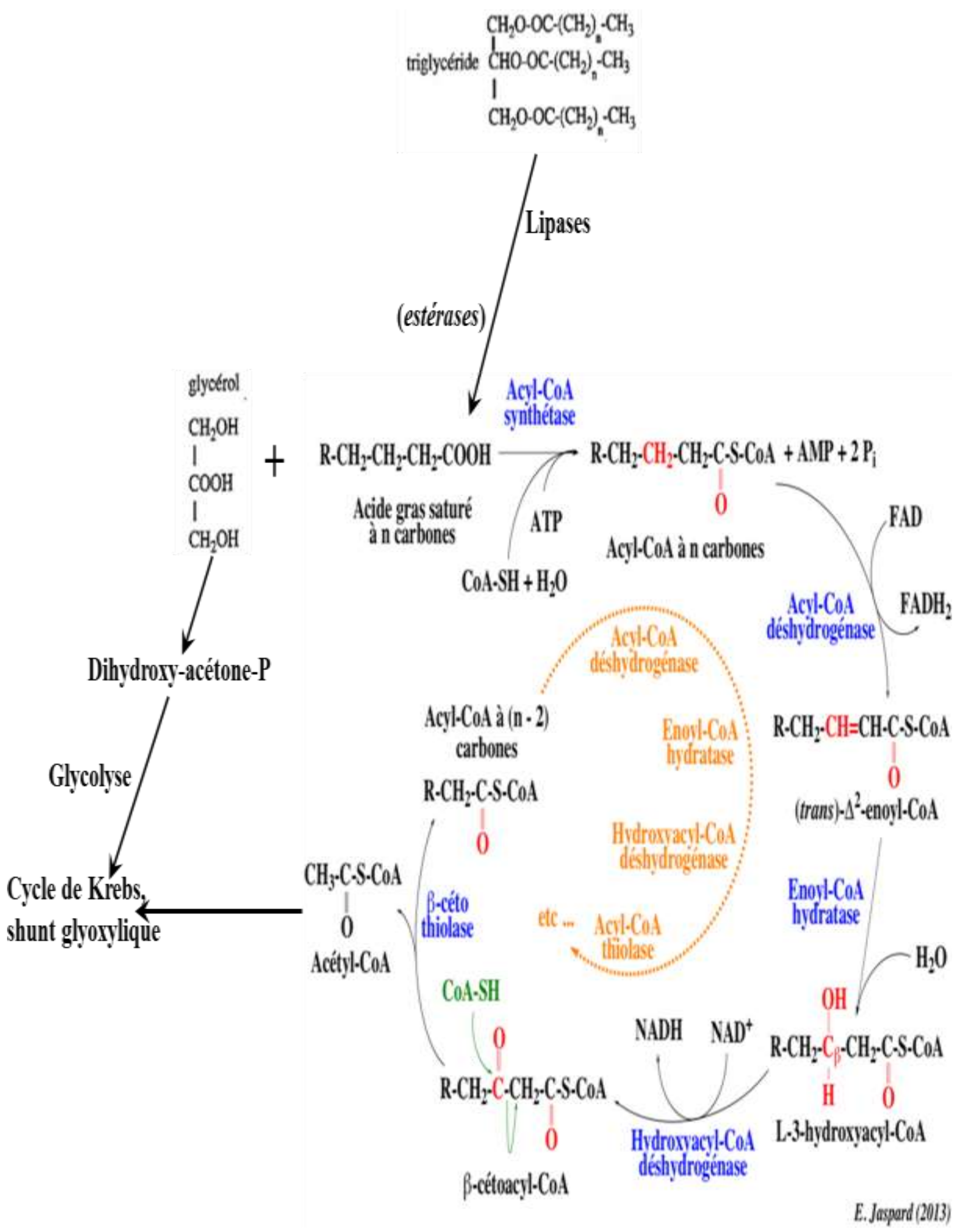


**Figure 12**: β-oxidation of fatty acids.

### 2.2.9. Protein catabolism

Proteins are high molecular weight organic compounds made up of amino acids linked together by peptide bonds. Numerous microbial proteases (generally extracellular) exist, with varying degrees of specificity: collagenases, gelatinases, etc. They act on both proteins and oligopeptides. They break down the protein molecule into polypeptide fragments, each composed of only a few amino acids. The best-known proteolytic species belong to the bacterial genera *Clostridium*, *Bacillus*, *Pseudomonas*, … as well as to many fungal genera.

Peptideses hydrolyze polypeptides and break them down into their constituent subunits, amino acids. The entry of amino acids depends on the presence of numerous and varied permease systems. Peptides are of two types, endopeptidases and exopeptidases, depending on their mechanism of attack on the polypeptide chain. Exopeptidases are themselves subdivided into two categories:

- **Aminopeptidases** begin their action at the free -$NH_2$ end of the polypeptide and their activity often depends on the presence of metal ions.
- **Carboxypeptidases** begin their attack at the free –COOH end of the polypeptide. The activity of these different enzymes leads to the release of di- and tripeptides, which are then hydrolyzed into amino acids.

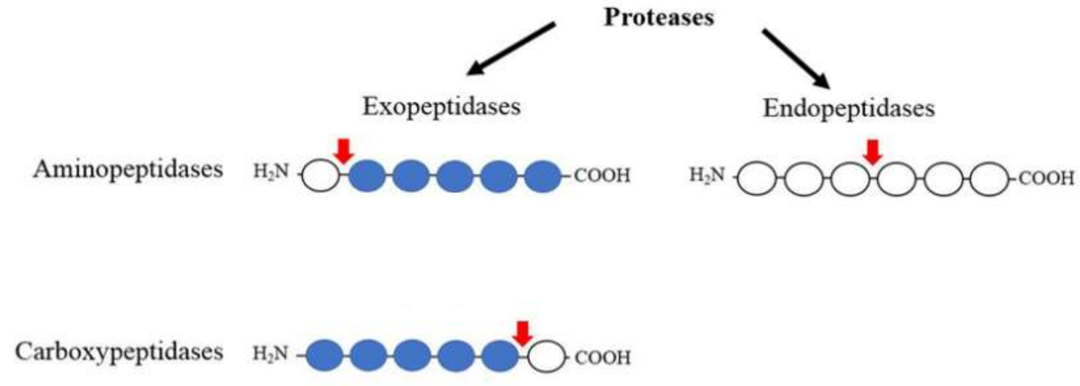


**Figure 13:** Mode of attack on the polypeptide chain.

### 2.2.10. Amino acid catabolism

There are two main pathways: deamination and decarboxylation.

### 2.2.10.1. Deamination

***Oxidative deamination***

It leads to the formation of an iminoacid (molecule possessing both a COOH (carboxyl) functional group and a >C=N– (imine) functional group which is then hydrolyzed into ammonia and α-keto acid: it involves flavin coenzymes (FAD).

***Non-oxidative deamination***

There are three types:

- **Desaturating deamination** produces ammonia and an unsaturated acid (example: aspartate is transformed into fumarate).
- **Deamination by dehydration** is specific to hydroxylated amino acids (serine) and is exclusively microbial. Ammonia and a ketone acid are formed. Cysteine degradation occurs through a similar reaction, but with the release of SH2 (cysteine sulfhydrase).
- **Reductive deamination** consists of a reduction of the amino acid into the corresponding saturated acid, with the formation of ammonia.
- **Coupled deamination (Stickland reaction)**. This is a coupled redox reaction between two amino acids, one acting as a hydrogen acceptor, the other as a donor.
- 

The acids produced by deamination enter the pathways of carbohydrate metabolism: pyruvate (alanine, glycine, serine, cysteine…), acetyl-CoA (leucine, isoleucine, lysine…), oxaloacetate (aspartate)…

### 2.2.10.2. Decarboxylation

Decarboxylases act on amino acids to form $CO_2$ and an amine. An acidic environment favors the formation of decarboxylases, while an alkaline environment stimulates the formation of deaminases.

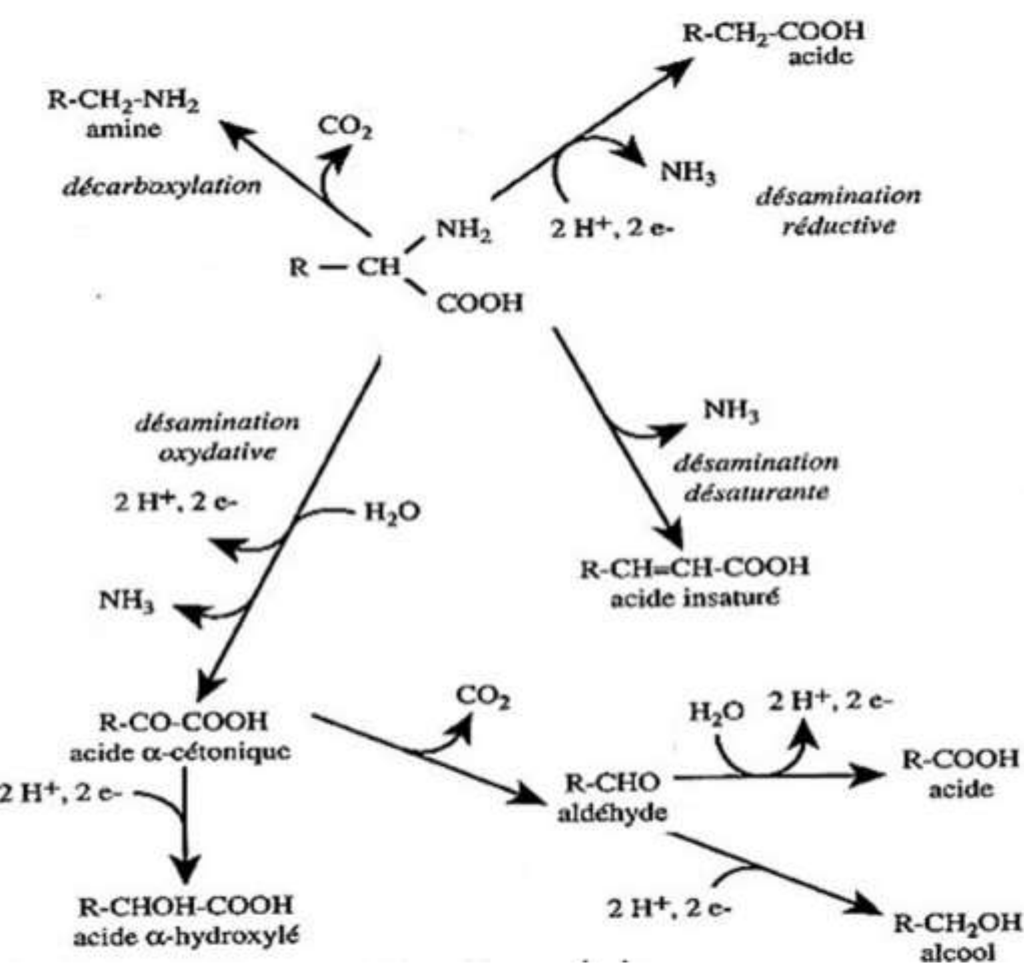


**Figure 14:** Degradation of amino acids.

### 2.2.11. Degradation of other sugars

- ***Pentoses***

The degradation of pentoses has been extensively studied in Enterobacteriaceae and Lactobacilli. Regardless of the pentose metabolized, its degradation results in the formation of D-xylulose-5-phosphate, which is then metabolized either via the hexose monophosphate pathway (pentose cycle) or the pentose phosphate pathway (heterolactic bacteria pathway), with the involvement of phosphoketolase. Depending on the starting pentose, isomerases, transketolases, and transaldolases are involved before yielding xylulose-5-phosphate. Xylose assimilation in bacteria involves an isomerase.

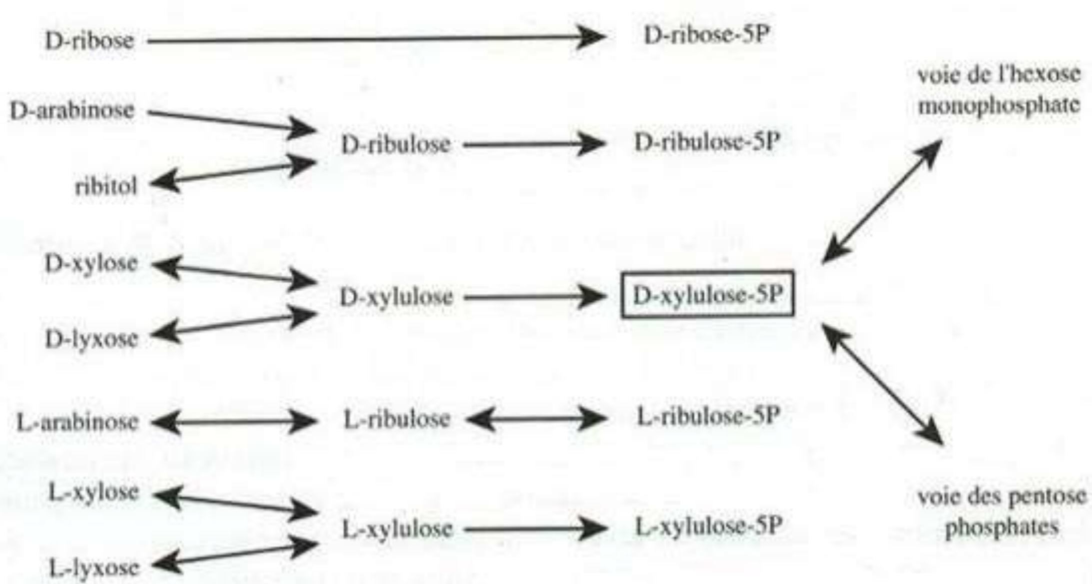


**Figure 15: General diagram of pentose metabolism.**

***Fructose***

Fructose can be either oxidized to 5-keto-D-fructose by D-fructose-NADP-5 oxidoreductase (Acetobacter cerinus, a strictly aerobic bacterium), or phosphorylated to fructose-1 (Escherichia coli, Zymomonas, Clostridium) or, more rarely, to fructose-6P. The first phosphorylation is followed by a second, resulting in fructose-1,6-bisphosphate, which is then degraded by glycolysis.

***Mannose***

Mannose can be catabolized by two different mechanisms: a cyclic mechanism and a non-cyclic mechanism. Both mechanisms exist for the D isomer, while the L isomer appears to be catabolized only by the non-cyclic mechanism.

In the cyclic mechanism (Aerobacter aerogenes), D-mannose is phosphorylated to mannose-6P, which is then converted to fructose-6P (subsequently metabolized by glycolysis). The phosphorylation of mannose occurs through the transfer of phosphate from glucose-6P to mannose. Glucose-6P is then regenerated either by isomerization of mannose-6P to fructose-6P or by direct phosphorylation of glucose, via a glucokinase. The utilization of L-mannose involves the non-cyclic mechanism. L-mannose is first converted to L-fructose by an isomerase. Fructose is then phosphorylated to fructose-1P, which is cleaved into dihydroxyacetone phosphate and L-glyceraldehyde, which is metabolized by glycolysis.

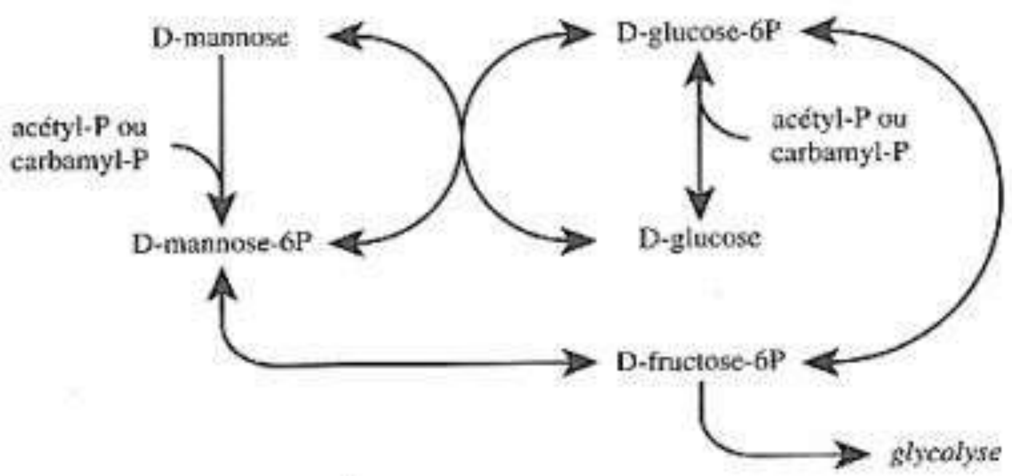


**Figure 16. Cyclical metabolism of mannose.**

***Sucrose***

Sucrose is first hydrolyzed into glucose and fructose by invertase present in many yeasts (Saccharomyces *cereviciae*), numerous molds(Aspergillus niger) and many bacteria (Clostridium pasteurianum). Glucose and fructose are degraded via the previously described pathways. Sucrose is hydrolyzed outside the cell in yeasts and molds. In many bacteria (lactic acid bacteria, Bacillus subtilis), sucrose is transported into the cell as sucrose-P and then hydrolyzed into glucose-6-P and fructose. In various bacteria (Bacillus subtilis, Zymomonas), there is also a levane sucrase that contributes to levane synthesis.

***Lactose and galactose***

Many microorganisms possess β-galactosidase: yeasts (Candida), molds (Aspergillus), and bacteria (E. coli, Lactobacillus, Bacillus). After lactose hydrolysis, the resulting glucose is broken down by one of the previously described pathways. Galactose, particularly in yeasts, is broken down by the Leloir-Kalchar pathway.

Galactose resulting from the hydrolysis of lactoseis converted into glucose-6P by a series of three reactions:

- He isFirst of allphosphorylated by ATP to galactose-1P under the action of galactokinase, metabolite directly usable by the cell after isomerization into glucose-6P.
- Phosphogalactose-uridyl transferase catalyzes the transfer of galactosyl from galactose-1P to UDP-glucose with the release of glucose-1P.
- The third reaction, catalyzed by UDP-galactose-4 epimerase, is an epimerization of galactosyl to glucosyl by inversion of the configuration of the hydroxyl in position 4.

In Escherichia coli, lactose metabolism depends on a specific permease and uses the Leloir pathway, as in yeast. In Lactobacillus casei, lactose is phosphorylated by a phosphotransferase system to lactose-P, which is cleaved within the cell into glucose and galactose-6-phosphate; metabolism occurs via the tagarose pathway. The tagarose pathway is also used in Staphylococcus aureus for the metabolism of lactose and galactose.

Galactose-6P → tagarose-6P

Tagarose-6P + ATP → tagarose-1,6-diP + ADP

Tagarose-1,6-diP → glyceraldehydes-3P + dihydroxyacétone-P

***Maltose***

It is generally hydrolyzed into 2 glucose molecules by maltase (or glucoamylase). In E. coli, it is metabolized via transglycosylation.

### 2.2.12. Alcohol Catabolism

***Glycerol degradation***

Glycerol catabolism has been studied in Enterobacteriaceae, Lactobacilli, acetic acid bacteria, and Clostridium butyricum. Glycerol is degraded, particularly in acetic acid bacteria, by two pathways (Figure 17). Acetobacter suboxydans, which lacks a Krebs cycle, can nevertheless metabolize glycerol. This bacterium is used for the production of dihydroxyacetone, an intermediate in glycerol degradation. Enterobacteriaceae catabolize glycerol by converting it to glyceraldehyde-3-phosphate (3-P), which is then degraded by glycolysis. This process is purely fermentative. Glycerol catabolism in Escherichia coli

involves a glycerol kinase that produces α-glycerophosphate, which is further converted to dihydroxyacetone phosphate.

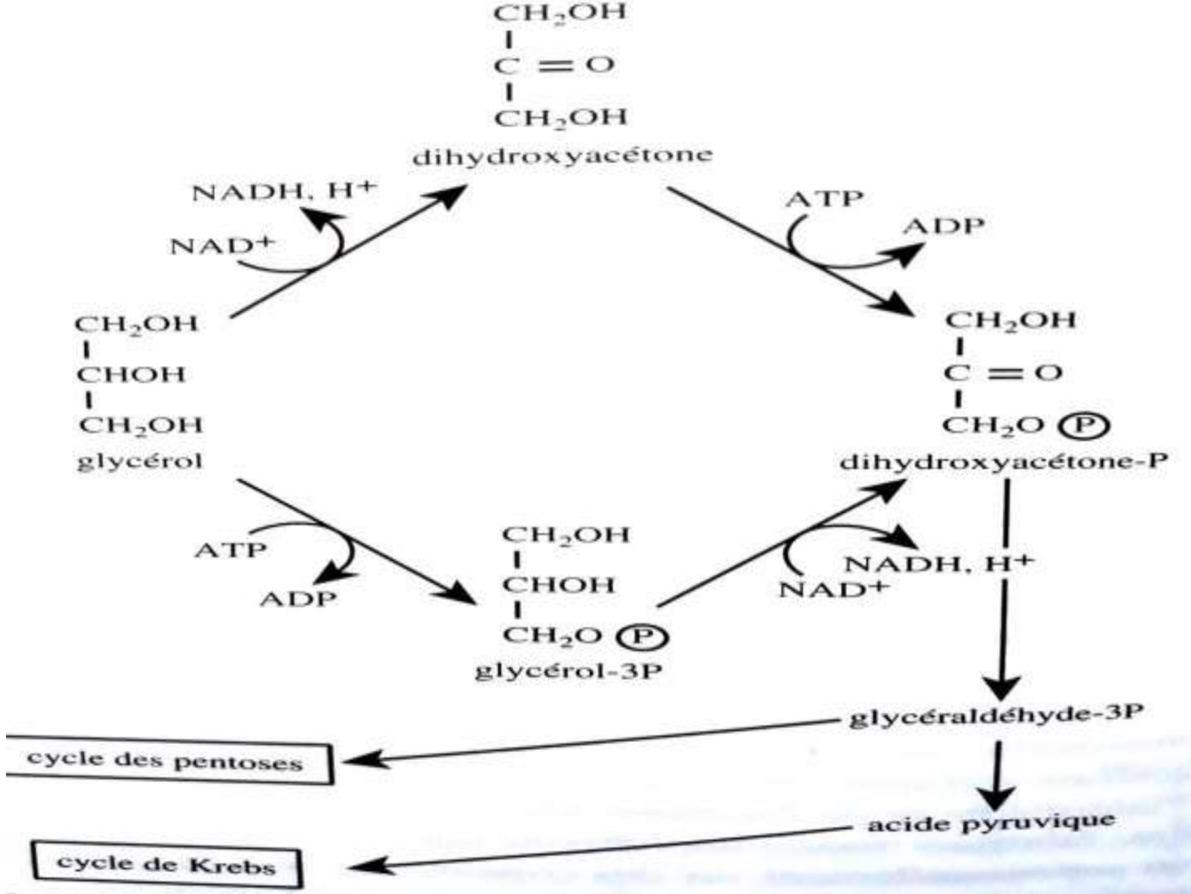


**Figure 17: Glycerol Catabolism**

***Ethanol degradation***

Ethanol can be completely degraded into $CO_2$ and $H_2O$, as in certain yeasts, or it can be transformed into acetic acid. In both cases, the first step leads to the formation of acetaldehyde. In the case of yeasts, acetaldehyde is incorporated into the Krebs cycle by oxidation to acetyl-CoA. This degradation is aerobic. In the case of acetic acid bacteria, acetaldehyde is directly transformed into acetic acid. This fermentation (the basis of vinegar production) is aerobic. Some acetic acid bacteria can then transform the acetic acid into $CO_2$ and $H_2O$.

### 2.2.13. Microbial Fermentations

Fermentation is a process that helps break down large organic molecules into simpler molecules through the action of microorganisms. For example, yeast enzymes convert sugars and starches into alcohol, while proteins are converted into peptides or amino acids. Fermentation is a ATP-generating process (Table II) in which organic compounds act as both electron donors (becoming oxidized) and electron acceptors (becoming reduced). Carbohydrates are the primary substrates of fermentation. Some compounds belonging to other chemical classes can also be fermented; organic acids, amino acids, purines, and pyrimidines.

**Table II:** The energy yield of certain microbial fermentations.

| **Fermentation** | **Energy** |
|---|---|
| **Alcoholic fermentation** | 2 mol ATP/mol glucose |
| **Propionic fermentation (glucose)** | 4 mol ATP/mol glucose |
| **Propionic (lactate) fermentation** | 0.3 mol ATP/mol lactate |
| **Amino acid fermentation** | 0.3 mol ATP /mol amino acids |
| **Homolactic fermentation** | 2 mol ATP/mol glucose |
| **Heterolactic fermentation** | 1 mol ATP/mol glucose |
| **Heterolactic fermentation of *Bifidobacterium*** | 2.5 mol ATP/mol glucose |
| **Mixed acid fermentation** | 2.5 mol ATP/mol glucose |
| **Butanediol Fermentation** | 2.5 mol ATP/mol glucose |
| **Butyric fermentation** | 3 mol ATP/mol glucose |

Fermentation is a natural way to enhance the vitamins, essential amino acids, antinutrients, proteins, appearance, flavor, and aroma of food. Microbial action on food ingredients tends to ferment the food, resulting in desirable biochemical changes that significantly alter the food. Depending on the enzymatic makeup of the bacterial species, different fermentation products will be obtained from pyruvate (Figure 18); it can also be completely oxidized. Many fermentations have industrial or diagnostic applications.

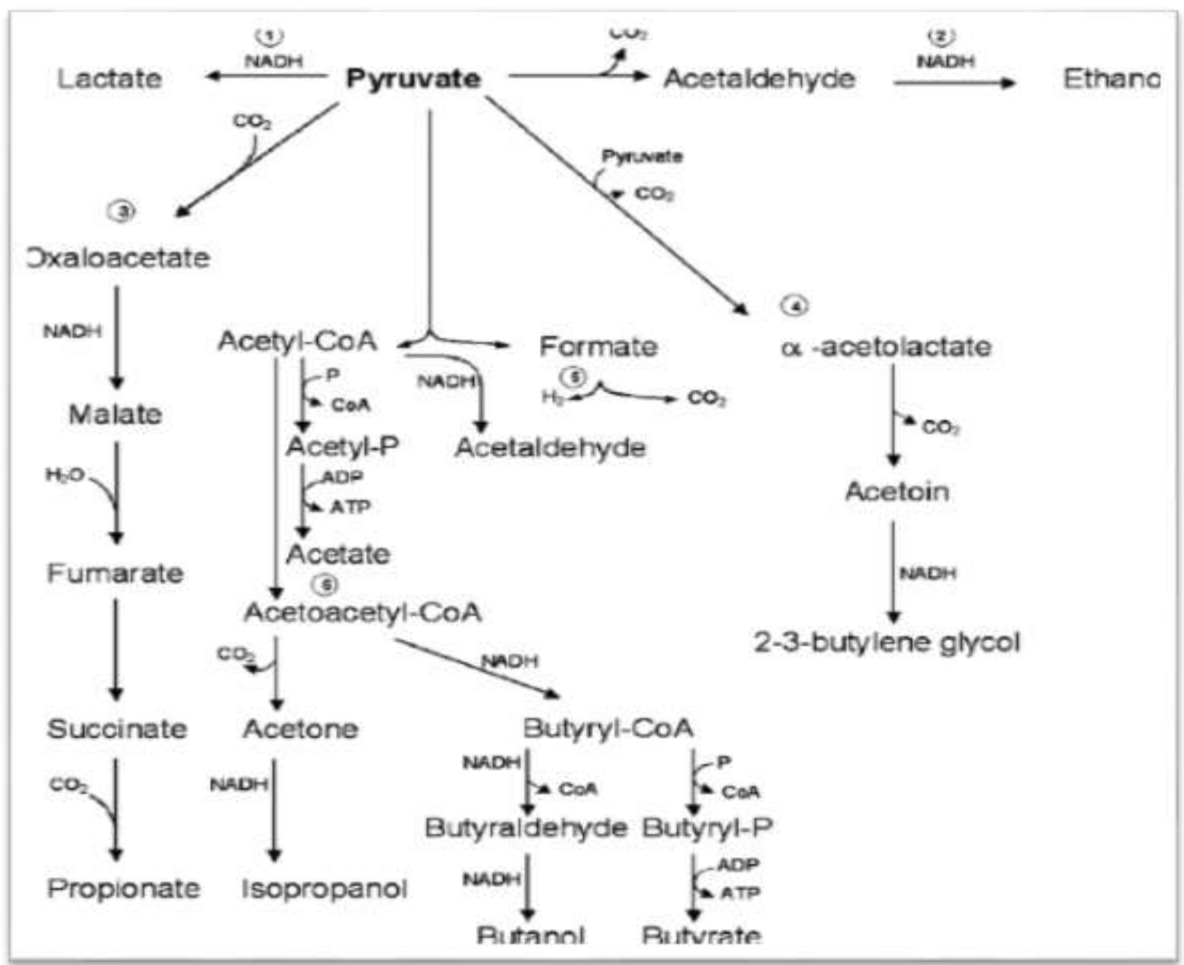


**Figure 18**: Microbial fermentations linked to pyruvate.**(1)**(1) Lactic acid bacteria (*Bacillus*, *Streptococcus*, *Lactobacillus*). (2) Yeasts, *Zymomonas mobilis*. (3) *Propionibacterium*. (4) *Enterobacter*, *Serratia*. (5) Enterobacteriaceae (*Escherichia*, *Salmonella*, *Proteus*).

**2.2.12.1. Lactic fermentation**

In the metabolism of lactic acid bacteria, lactic acid fermentation can synthesize a variety of organic acids, including lactic acid. This fermentation is divided into homolactic and heterolactic fermentation. Lactobacillus carries out homolactic fermentation, while Leuconostoc and Bifidobacterium carry out heterolactic fermentation. The homolactic and heterolactic fermentation pathways of sugars are shown in the Figure. Lactic acid is an essential organic acid as an industrial raw material with several applications in agriculture, food, pharmaceuticals, medicine, chemical industries, environmental protection and cosmetics (Figure 19).

### 2.2.12.2. Homolactic fermentation

The first part of the homolactic fermentation of glucose to lactate is the oxidative pathway, using glucose as a carbon source to produce pyruvate and reducing equivalents via glycolysis. The reducing equivalents accumulate as NADH. In the second part of this fermentation, the reducing equivalents are reoxidized, and pyruvate acts as an electron acceptor and is reduced to lactate by lactate dehydrogenase. This second reaction utilizes NADH, which regenerates NADH.

### 2.2.12.3. Heterolactic fermentation

In lactic acid bacteria of the heterolactic family, glucose can be broken down into lactate, ethanol, acetate and $CO_2$. The term heterolactic fermentation is explained by the presence of other compounds alongside lactic acid.

### 2.2.12.4. Mixed acid fermentation

Sugar fermentation occurs via the glycolysis pathway, resulting in a wide variety of fermentation products, including lactic acid, acetic acid, succinic acid, formic acid, and ethanol (Figure 20).

### 2.2.12. 5. Glycolic butylene fermentation

Glycolic butylene fermentation is accompanied by the production of acetoin and 2, 3-butanediol. A unique fermentative metabolic characteristic of Enterobacter, Serratia, and certain Erwinia species is the formation of a neutral product (butanediol). The metabolic properties of members of the Enterobacteriaceae family are useful for characterizing and distinguishing them. Acetoin, upon oxidation in air, yields diacetyl (responsible for the buttery taste and produced during cream ripening). Butanediol can be converted into butadiene and used in the synthesis of synthetic rubber (Figure 20).

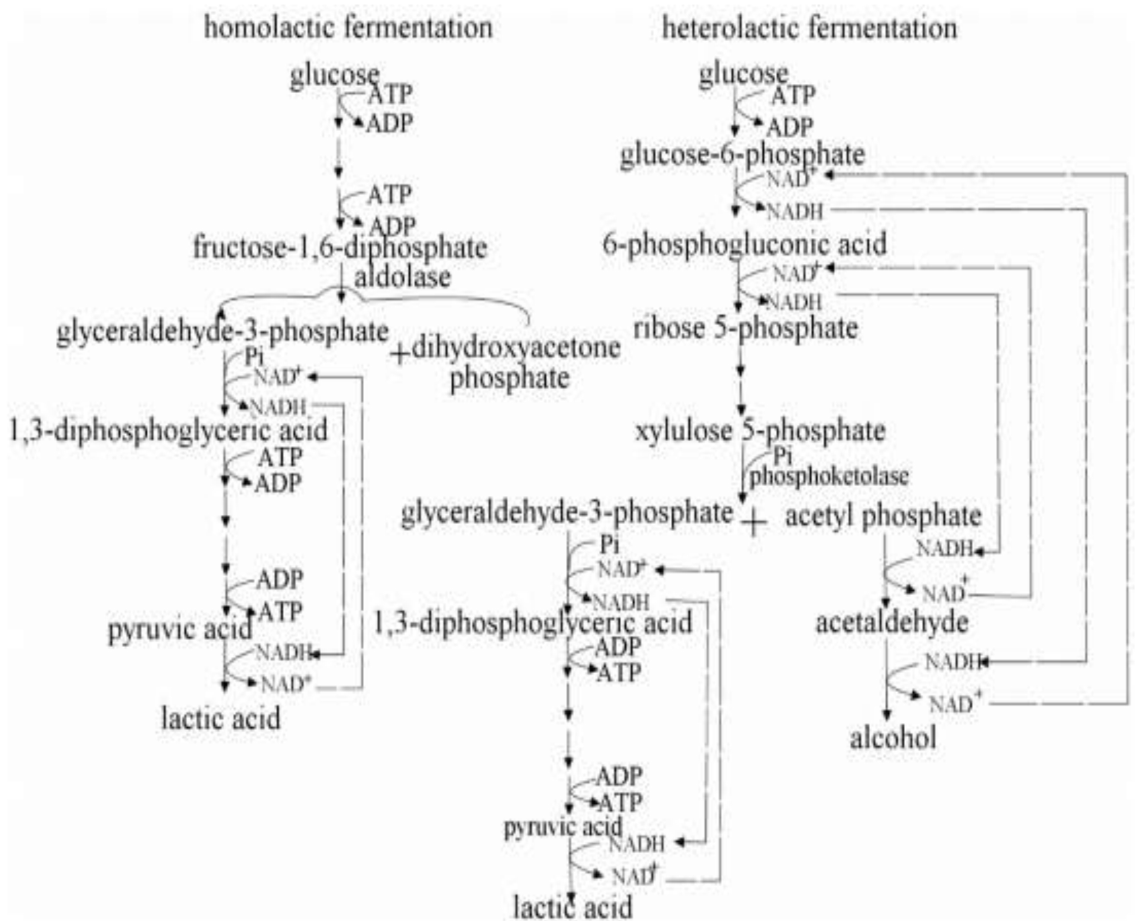


**Figure 19.** Homolactic and heterolactic fermentation

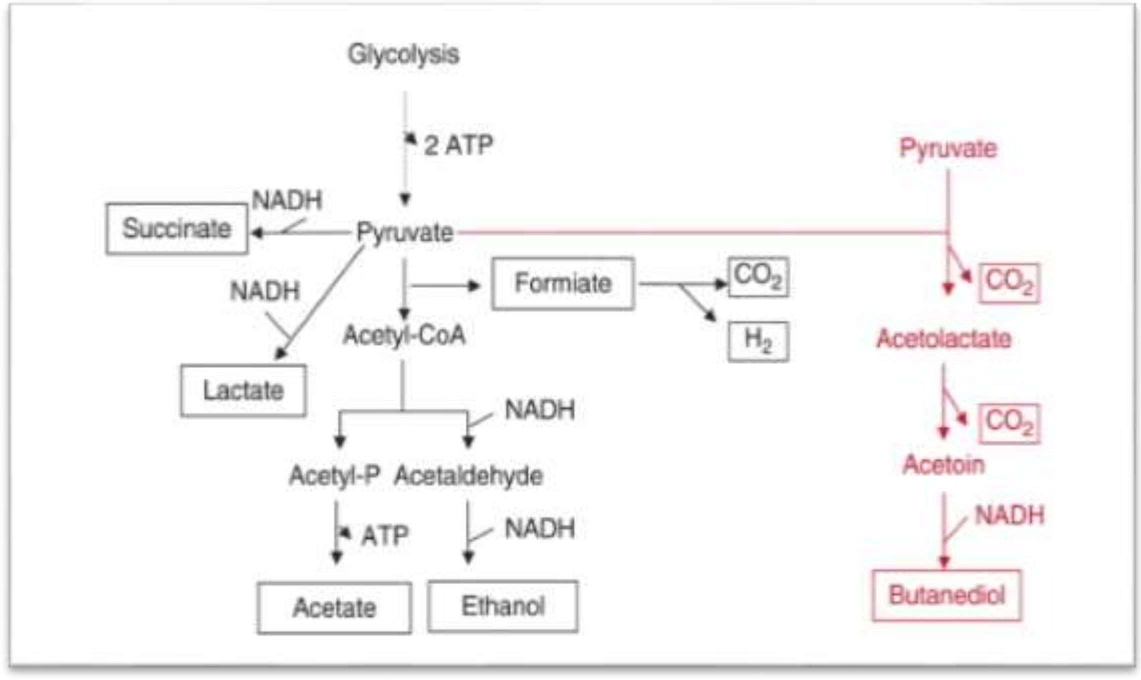


**Figure 20:** Mixed acid and glycolic butylene fermentation.

### 2.2.12.6. Acetic fermentation

Food-grade acetic acid can be produced by the two-step vinegar process. The first step is the production of ethanol from a carbohydrate source such as glucose. This is carried out at 30-32°C using the anaerobic yeast *Saccharomyces cerevisiae*.

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2CH_3CH_2OH$$

The second step is the oxidation of ethanol to acetic acid. Although various bacteria can produce acetic acid, only members of the Acetobacter genus, such as *Acetobacter aceti*, are used commercially. This fermentation is an incomplete oxidation because the reducing equivalents generated are transferred to oxygen and not to carbon dioxide.

$$2CH_3CH_2OH \longrightarrow O_2 + 2CH_3COOH + 2H_2O$$

### 2.2.12.7. Propionic fermentation

Propionic fermentation is carried out by several bacteria belonging to the genus Propionibacterium and the species Clostridium propionicum. During propionic acid fermentation, sugar and lactate can be used as initial substrates. When sugar is available, these bacteria use glycolysis to produce pyruvate. Pyruvate is carboxylated to oxaloacetate, then reduced to propionate via malate, fumarate, and succinate. Other products of propionic fermentation are acetic acid and $CO_2$ in varying proportions. This process corresponds to the secondary fermentation of certain cooked-curd cheeses (Gryurère and Emmental). Propionic acid is a natural carboxylic acid that contributes to the flavor, while $CO_2$ is responsible for the holes. Propionic acid is widely used as a food preservative and stabilizer in the food industry (Figure 21).

### 2.2.12.8. Butyric fermentation

Butyric fermentation carried out by Clostridium butyricum, it gives rise to the following by-products (butyrate). This is the typical fermentation of spoiled canned goods, characterized by a bad odor, gas production, and acidity. *Clostridium butyricum*, which is often found in large quantities in silage, can be found in milk and cause lactic acid to ferment into butyric and hydrogen acids in wheels of Gruyère or Emmental cheese, causing them to burst (Figure 21).

Most butyric acid is consumed in the manufacture of plastics. A certain amount of butyric acid is used to make herbicides. It is also used as an intermediate in pharmaceuticals, emulsifiers, and disinfectants, as a leather tanning agent, and as a sweetening agent in gasoline. It is used in the synthesis of butyrate ester perfumes and in the manufacture of esters, some of which serve as the basis for artificial flavoring ingredients in certain liqueurs, soda syrups, and candies. Another use is as a food additive in the flavorings of butter, cheese, butterscotch, caramel, fruits, and nuts. Butyric acid is also used in preserving very moist wheat grains to prevent fungal spoilage.

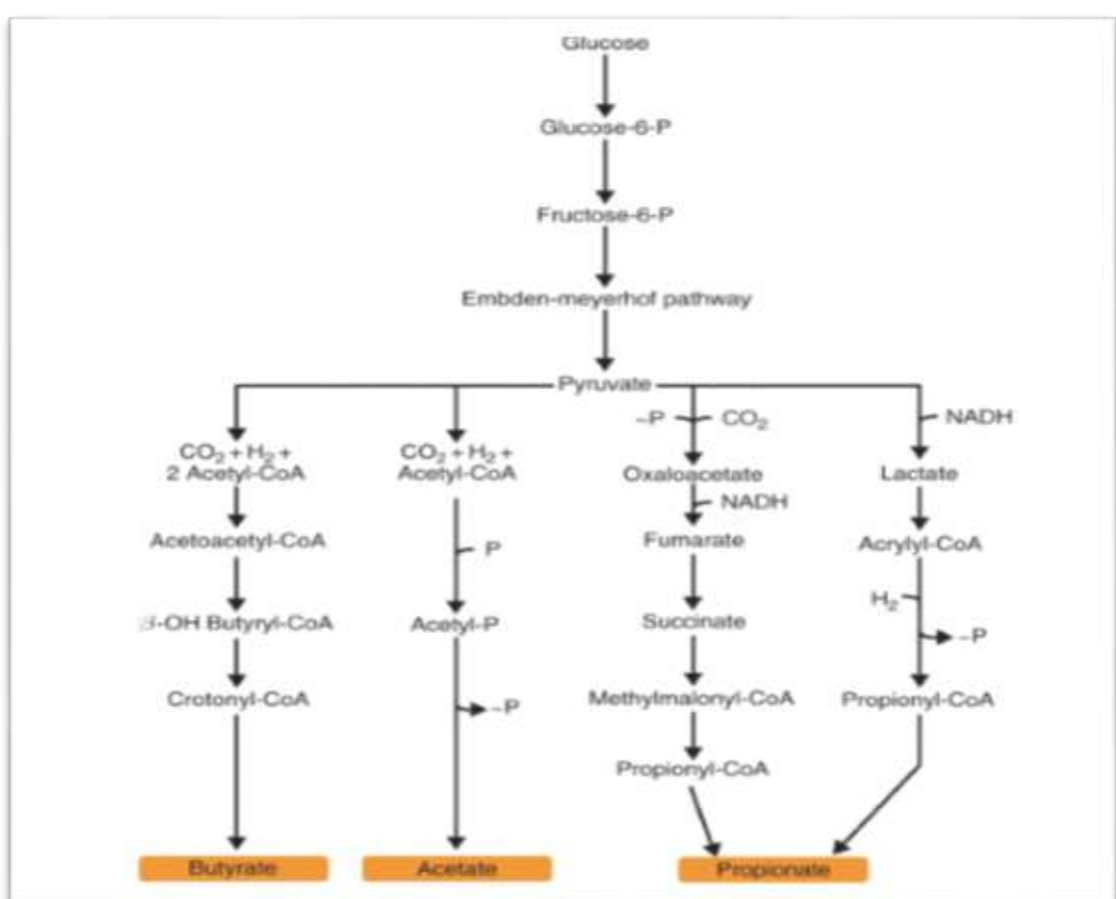


**Figure21**: Propionic and butyric fermentation.

### 2.2.12.9. Aceto-butylic fermentation

Acetobutylic fermentation is based on the culture of various *Clostridium* strains in carbohydrate-rich media under anaerobic conditions to produce butanol and acetone. Clostridium acetobutylicum is the organism of choice for the production of these organic solvents. These fermentations were not popular until very recently due to the availability of acetone and butanol from the petroleum industry. Today, these fermentations are generating considerable interest. However, the concentration of final products in these fermentations is quite low, and they are a type of mixed fermentation yielding a mixture of compounds such as butyric acid, butanol, and acetone (Figure 22).

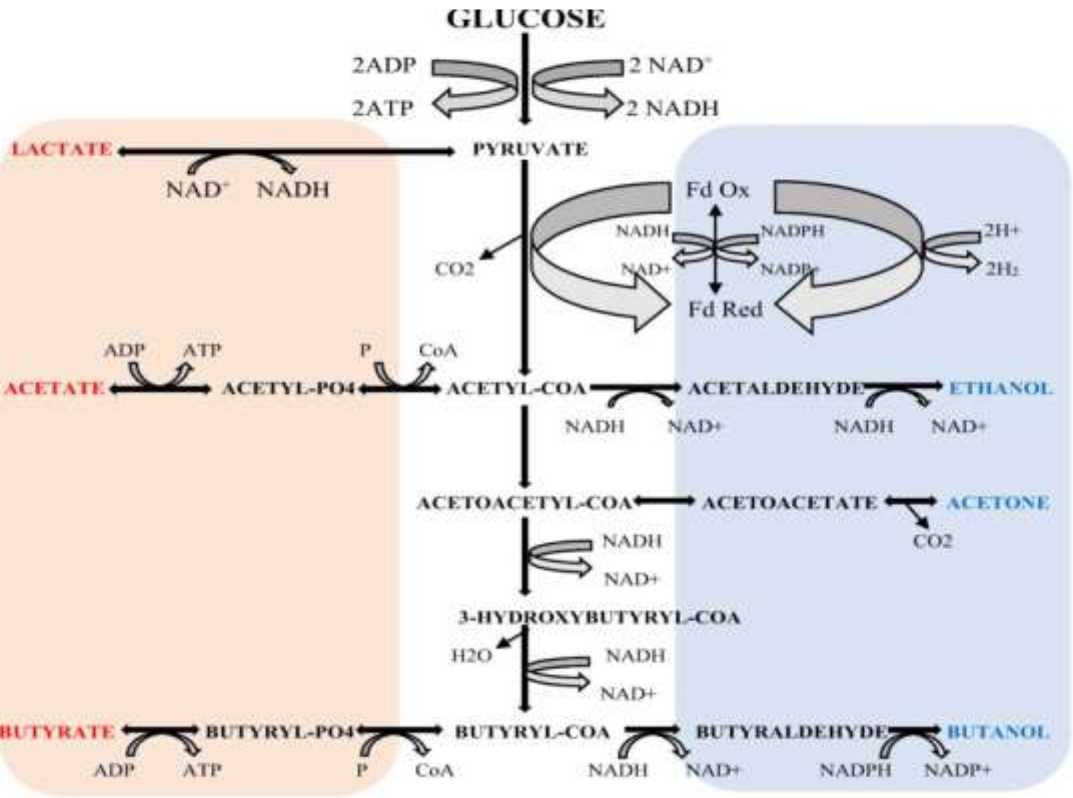


**Figure 22:** Acetob-butylic fermentation.

### 2.2.12.10. Kojic fermentation

Kojic acid is a fungal metabolic product produced by some *Aspergillus* species, particularly A. oryzae, which has the common Japanese name koji (Figure 23). Kojic acid was first isolated in 1907 by Saito from A. oryzae mycelia grown on steamed rice. In 1912, Yabuta named it kojic acid, and it was not until 1924 that he deciphered the correct molecular structure of this acid. Kojic acid is an inhibitor of bacterial and fungal growth and viral replication. Due to its antibacterial, antioxidant, and color-preserving properties, kojic acid is used in the cosmetics and food industries as a precursor to flavor enhancers (maltol and ethyl maltol), on cut fruit to prevent oxidative browning, to prevent fruits and vegetables from becoming stale, and in seafood and meats to preserve pink and red colors. Kojic acid is widely consumed in the Japanese diet, with the belief that it is beneficial to health.

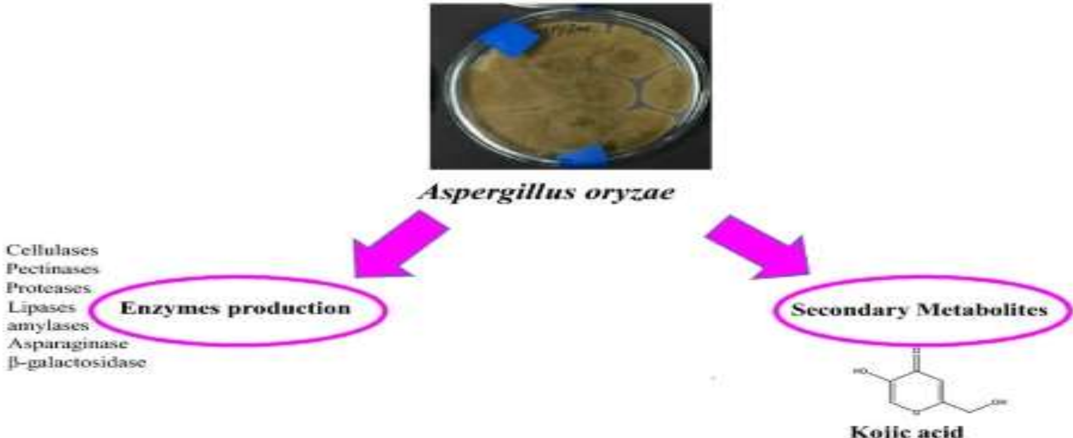


**Figure 23**: Some of the interests of *Aspergillus oryzae* in biotechnology.

### 2.2.12.13. Gluconic fermentation

Gluconic acid (pentahydroxycaproic acid, $C_6H_{12}O_7$) is the oxidation product of glucose, occurring naturally in plants, fruits, and other natural sources. The physiological D form of gluconic acid is generally formed by the microbial oxidation of glucose (Figure 24). Gluconic acid is commercially produced from fungal organic acids, from glucose or sucrose using selected strains of *Aspergillus niger*. Gluconic acid is a product of great interest for many applications.in various industrial sectors; such as detergents and pharmaceuticals (iron and calcium deficiency).

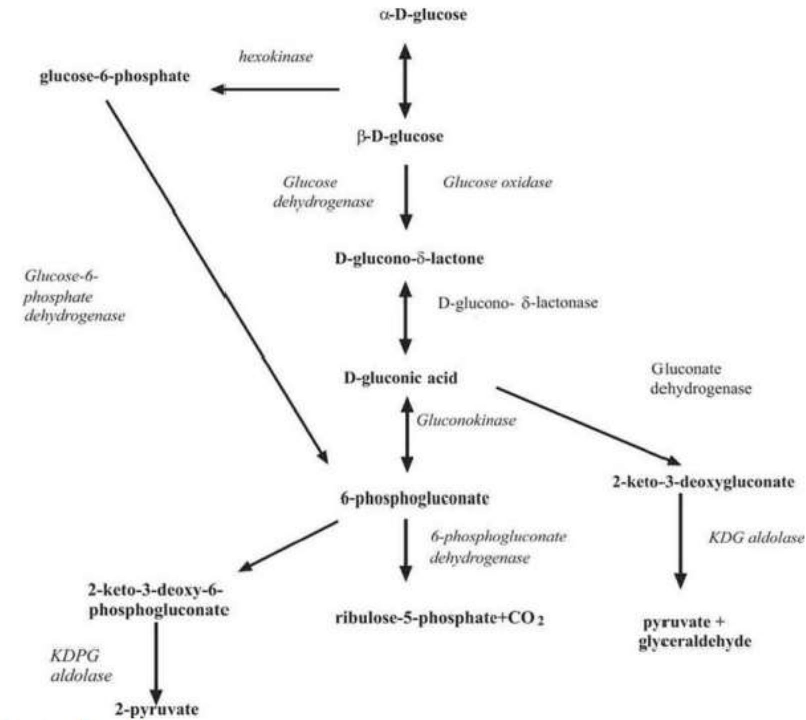


**Figure 24:** Gluconic fermentation.

### 2.2.12.14. Citric fermentation

Citric acid ($C_6H_8O_7$) is a constituent of citrus fruits, such as oranges and lemons, which were once the only industrial source of this acid. However, over a century ago, it was discovered that citric acid is a product of mold metabolism, particularly that of *Aspergillus niger* (the most efficient producer of citric acid). Indeed, citric acid produced by *Aspergillus niger* is widely used in food, as a food additive, and in the composition of detergents and cosmetics.

### 2.2.12.15. Oxalic fermentation

Oxalic acid and its salts are used as reagents in analyses, as well as in the synthesis of many organic compounds. Due to its high reducing power, it is used as a bleaching agent. *Aspergillus niger* is capable of producing a significant amount of oxalic acid using sucrose as a source of carbon and energy.

### 2.2.12.16. Itaconic fermentation

The metabolic pathway for itaconic acid production has been studied in *Aspergillus terreus*. Itaconic acid is produced from cis-aconitic acid, an intermediate in the citric acid cycle. Glucose from the extracellular environment is converted to pyruvate via glycolysis, followed by oxidative decarboxylation to generate acetyl-CoA. In the mitochondria, acetyl-CoA and oxaloacetate form citric acid via citrate synthase. Citric acid is dehydrated to cis-aconitic acid,

which is transported to the cytosol by a transporter and converted to itaconic acid by cis-aconitic acid decarboxylase (Figure 25). It is widely used for polymer production. Itaconic acid also exhibits some antimicrobial activity.

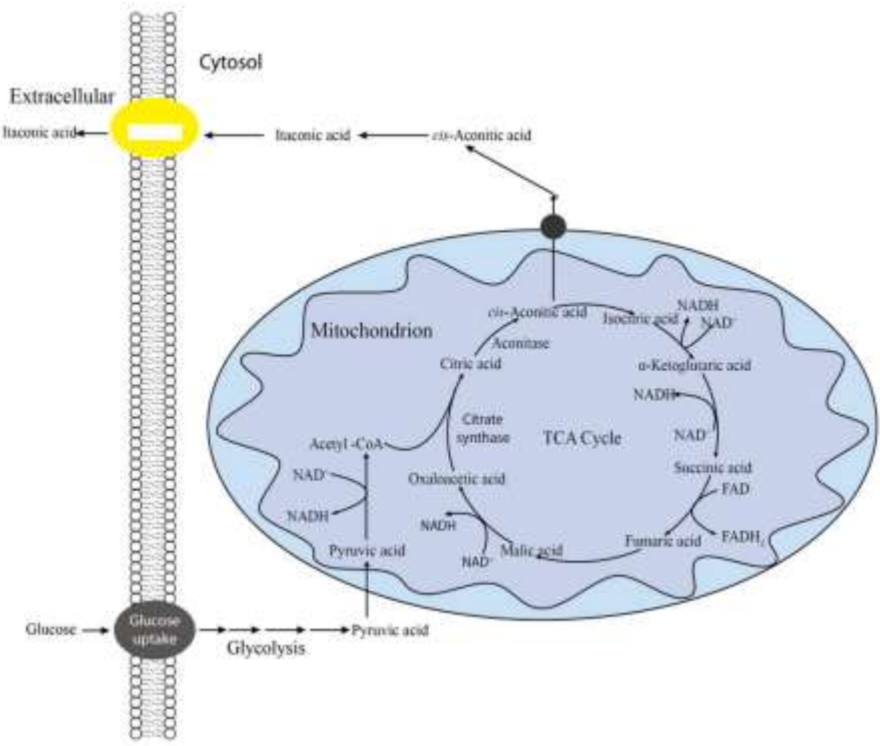


**Figure 25 :** Itaconic fermentation.

**2.2.13. Phytosynthesis**

In microbes, photosynthesis can be anxoygenic (does not produce oxygen) or oxygenic (produces oxygen). Cyanobacteria are microbes that exhibit oxygenic photosynthesis. This group, formerly called blue-green algae, includes unicellular forms and filamentous forms. The photosynthetic apparatus of cyanobacteria is located in intracellular membrane systems called thylakoids. These pigments capture light energy. These pigments are arranged in a highly organized manner to maximize the transfer of light energy into photosystem I (PS I), which absorbs light at 700 nm, and photosystem II (PS II), which absorbs light at 680 nm. Photosynthesis in algae is similar to that of cyanobacteria (Figure 26).

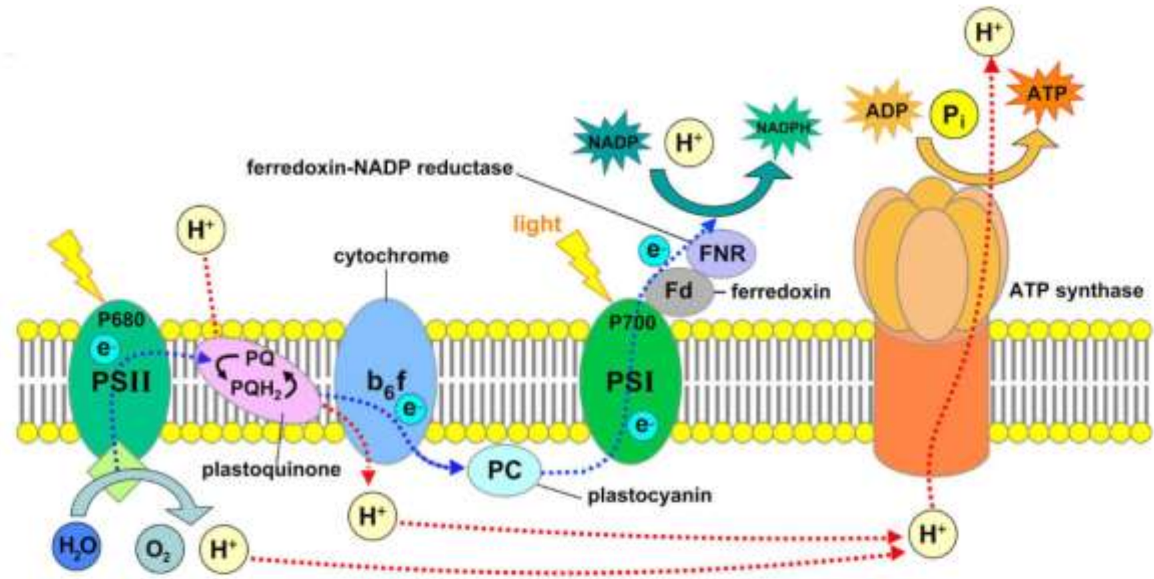


**Figure 26**: Photosynthesis in cyanobacteria.

### 2.2.13.1. Oxygen Photophosphorylation

Electron flow in cyanobacteria is initiated when PSI transfers light energy to chlorophyll P700. The molecule's reduction potential then becomes more negative. It can then donate an electron to another chlorophyll. The electron is then transferred to a ferredoxin and can follow one of two pathways:

- The cyclic pathway involves the movement of electrons through a series of electron carriers and the return to P700 with ATP generation after the formation of a transmembrane proton gradient. This process is called cyclic photophosphorylation and requires only one photosystem (Figure 27).
- In the non-cyclic pathway, ATP is formed when electrons are transferred at PS II or PS I. This process is called non-cyclic photophosphorylation. The non-cyclic pathway generates ATP and NADPH (Figure 27).

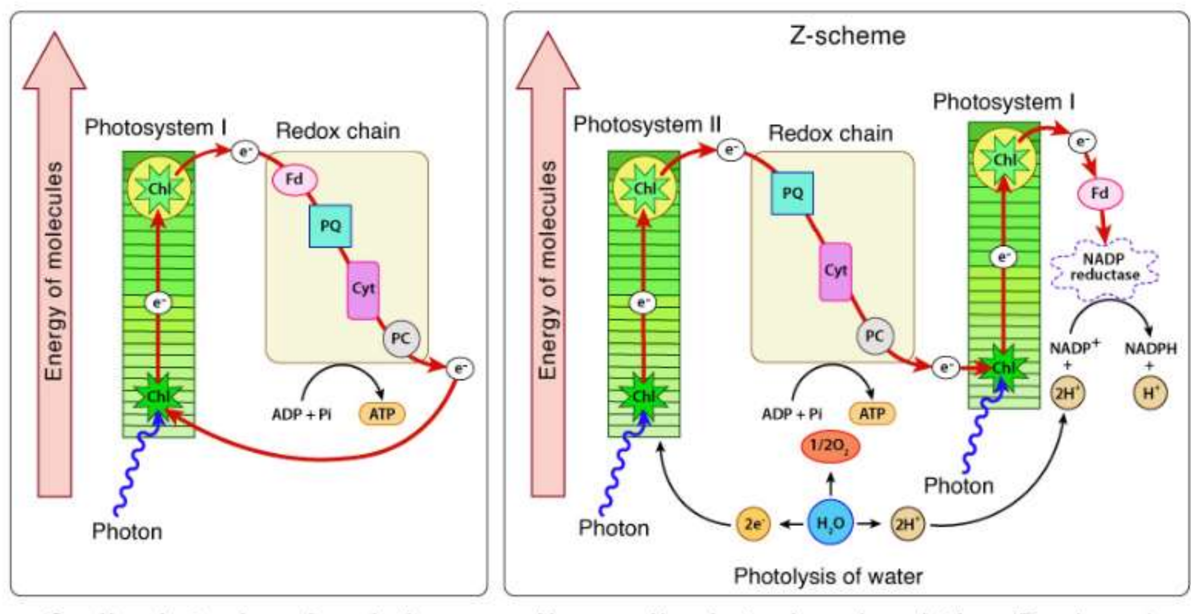


**Figure 27**: Cyclic and non-cyclic phosphorylation.

### 2.2.13.2. Anoxygenic photosynthesis

Green and purple bacteria differ from cyanobacteria in that most are strict anaerobes and do not use water as an electron source. These organisms use hydrogen (H), hydrogen sulfide (HS), and sulfur as electron donors and possess different photosensitive pigments (bacteriochlorophylls) that absorb light at long wavelengths: bacteriochlorophyll a (maximum absorption at 775 nm) and bacteriochlorophyll b (79 nm). These differences are thought to reflect the wavelengths available within their ecological niches. Neither group has photosynthetic ionization (PSII); therefore, they do not produce oxygen and NADPH through photosynthesis. Both exhibit cyclic electron transport, which can be used to generate ATP. These organisms are unable to synthesize NADPH directly through the photosynthetic movement of electrons.

### 2.2.213.3. Calvin Cycle

If $CO_2$ is used as the carbon source, cyanobacteria and purple bacteria use the Calvin cycle for carbon fixation. The overall reaction is:

$$6\ CO_2 + 18ATP + 12NADPH + 12H^+ 12H_2O \rightarrow glucose + 18ADP + 18P + 12NADP^+$$

To synthesize glucose, the cycle must complete six times.

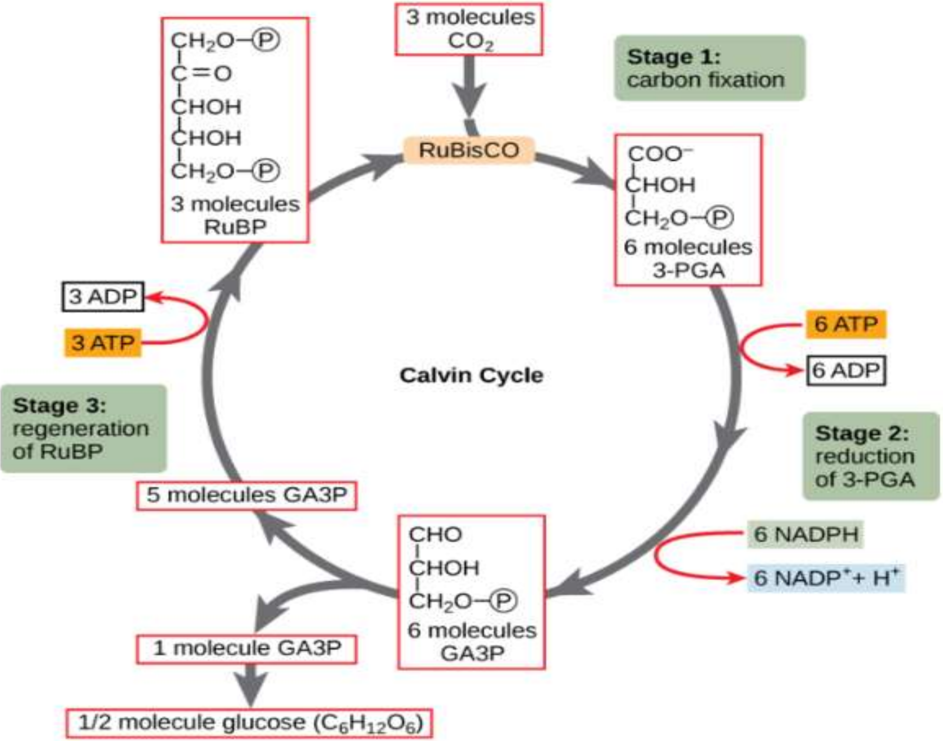


**Figure 28.** Calvin cycle.

### 2.2.14. Anabolism

#### 2.2.14.1. Fatty acid biosynthesis

Fatty acids are precursors to a variety of important building blocks such as phospholipids, sphingolipids, and sterols, serving as secondary metabolites and signaling molecules, or as protein binding sites. Since fatty acid breakdown produces a large amount of ATP and reducing equivalents, they also represent a suitable storage compound for energy and carbon. Fatty acids are stored as triacylglycerol (TAG) or wax esters. Initially, malonyl-CoA is formed by the carboxylation of acetyl-CoA in the presence of ATP. Coenzyme A is then exchanged for acyl carrier protein (ACP), yielding malonyl-ACP. ACP prevents the degradation of the growing fatty acid chain and its use for anabolic reactions. With malonyl-ACP, the first round of the fatty acid biosynthesis cycle begins with the initial condensation of malonyl-ACP with acetyl-CoA, producing acetoacetyl-ACP and free coenzyme A. The latter is then reduced to 3-hydroxybutyryl-ACP, dehydrated to 2-butenoyl-ACP, and further reduced to butyryl-ACP. Butyryl-ACP re-enters the next round of the cycle by condensation with malonyl-ACP.

Fatty acid synthesis stops when a certain chain length is reached, and acyl-ACP is used for membrane synthesis. The two reduction steps require two reduction equivalents, derived from nicotinamide adenine dinucleotide (NADPH).To metabolize fatty acids, they must be

activated into acyl-CoA esters. The acyl-CoA formed is consumed via β-oxidation. The degradation of acyl-CoA compounds occurs in a cycle that reverses the steps of fatty acid biosynthesis, resulting in the release of one acetyl-CoA unit in each cycle. β-Ketothiolase catalyzes the final step of the cycle, in which acetyl-CoA and acyl-CoA (reduced by two carbon atoms) are formed (Figure 29).

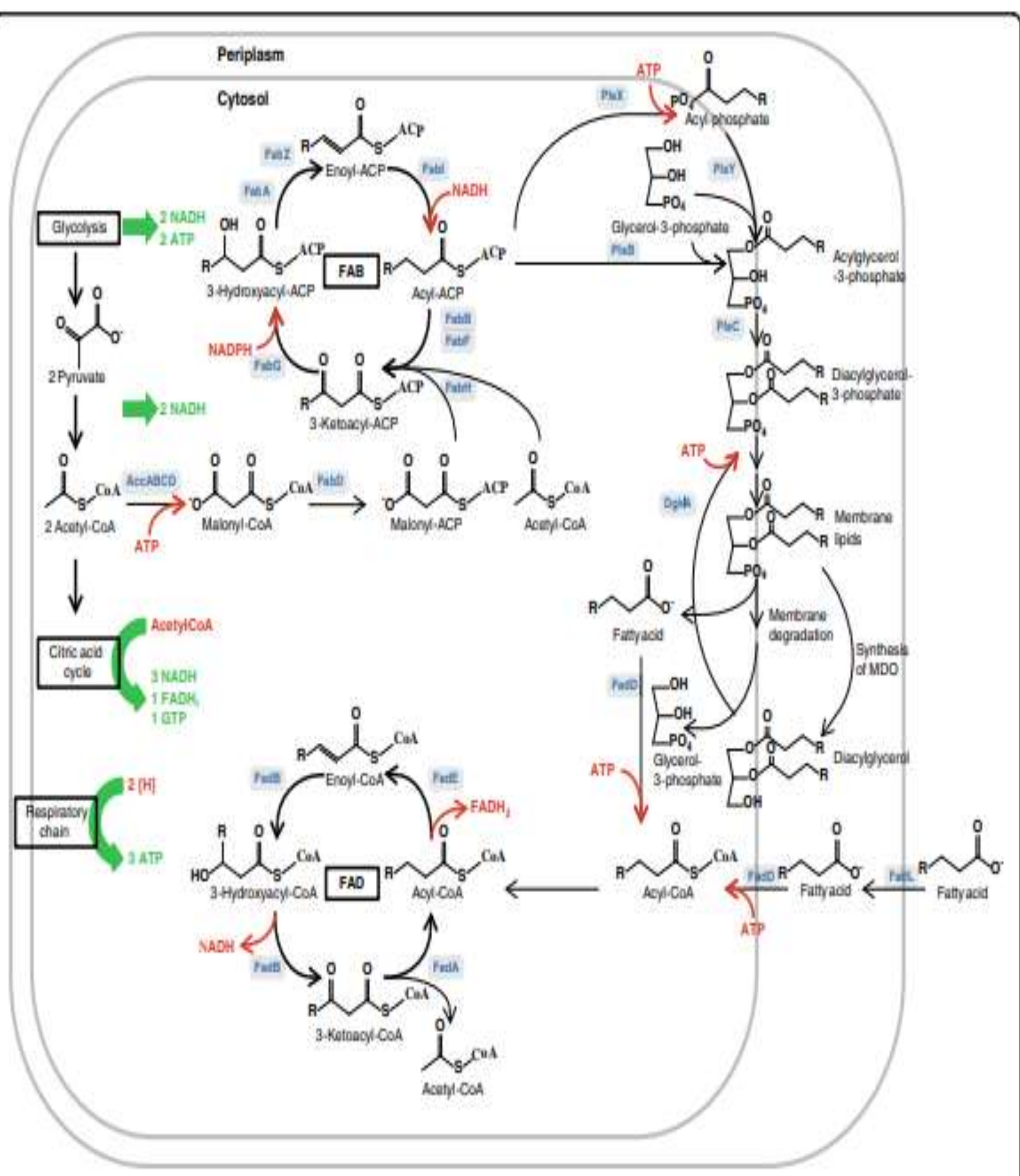


**Figure 29.** The breakdown and metabolism of fatty acids.

### 2.2.14.2. Phospholipid biosynthesis

*E. coli* possesses only three major phospholipid species in its membranes, making it one of the simplest organisms to study with respect to phospholipid biosynthesis. Phosphatidylethanolamine constitutes the majority of phospholipids (75%), with phosphatidylglycerol and cardiolipin making up the remainder (15–20% and 5–10%, respectively). Phospholipid biosynthesis is linked to glycolysis through the use of dihydroxyacetone phosphate (DHAP), formed from glycerol-3-phosphate via its reduction by NADH, catalyzed by glycerophosphate dehydrogenase (Figure 30).

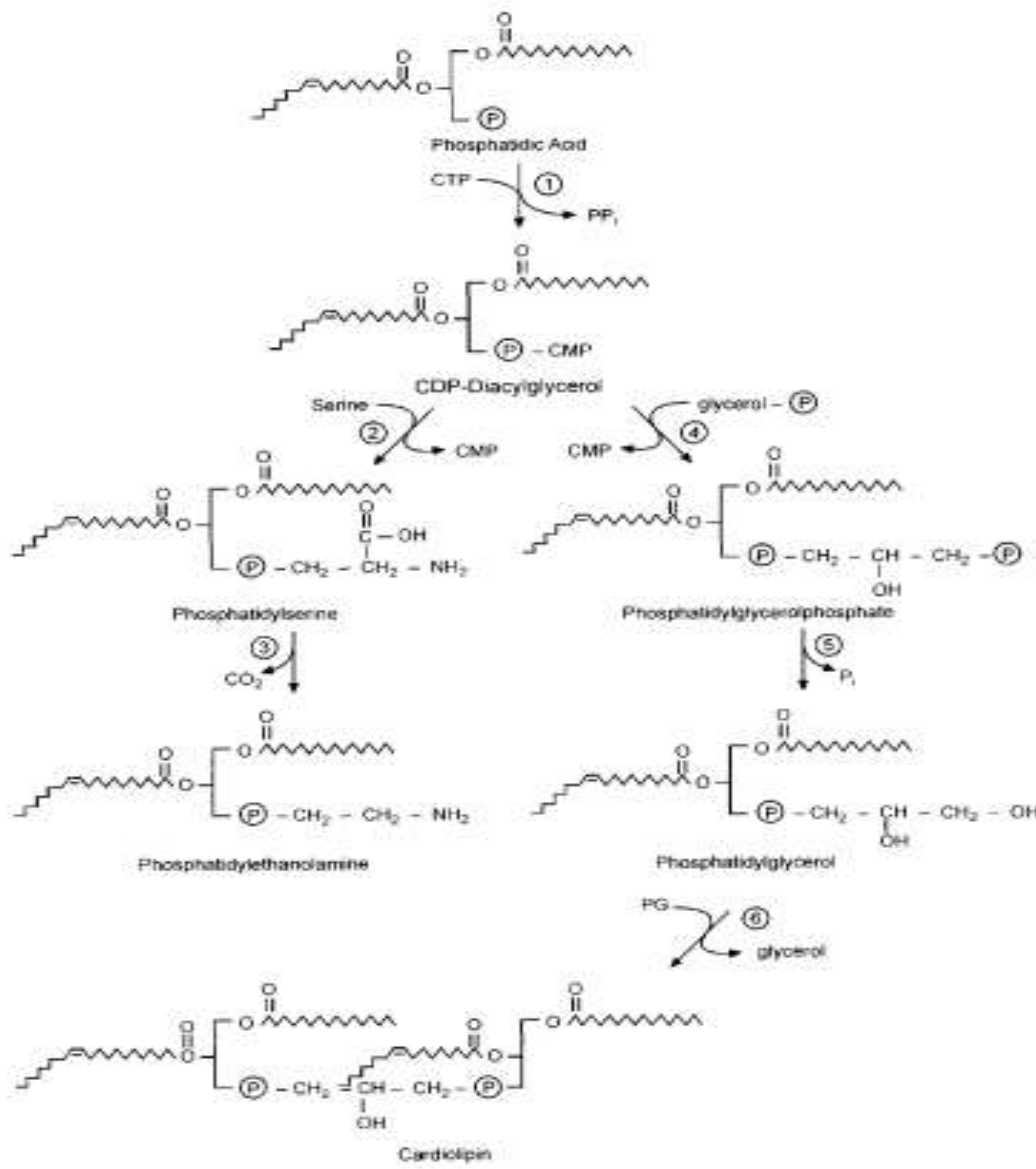


**Figure 30.** Synthesis of polar phospholipids.

### 2.2.14. 3. Amino acids anabolism

The amino acids synthesized in the cell are used, for the most part, for protein synthesis because numerous regulatory systems are present within the cell. The amino acid backbones are derived from acetyl-CoA, as well as from intermediates of the tricarboxylic acid cycle, glycolysis, and the pentose phosphate cycle. To make the process efficient and economical, the precursors for amino acid biosynthesis originate from a few main amphibolic pathways. Twenty amino acids are required for protein biosynthesis, and they are formed from the metabolic precursors listed in Table III. This table shows that only a few compounds serve as substrates in amino acid synthesis. The use of molecular nitrogen is only possible in a limited number of microorganisms.

**Table III.** Precursors used for amino acid biosynthesis.

| **Precursor** | **Amino acids** |
|---|---|
| Pyruvate | Alanine, valine, leucine |
| Oxaloacetate | Aspartate, asparagine, methionine, lysine, isoleucine, threonine |
| α-ketoglutarate | Glutamate, glutamine, arginine, proline |
| 3-phosphoglycerate | Serine, glycine, cysteine |
| PEP and erythrosis 4 phosphate | Phenyalanine, tyrosine, tryptophan |
| Ribose 5 phosphate | Histidine |