CHAPTER II:
ENERGETIC METABOLISM of MICROORGANISMS

2. Electron acceptors and respiratory types

2.1 Aerobic respiration

Traditionally, when the final electron acceptor is **molecular oxygen**, this process is referred to as respiration, and microorganisms that undergo this process are called **aerobic**.

There are various mechanisms of **aerobic respiration**, which can only occur under aerobic conditions. Microorganisms that possess only this type of system are referred to as "**strict aerobes**".

Microorganisms performing respiration possess an electron transport chain, also known as the "**respiratory chain**" or "**oxidative phosphorylation chain**", which is associated with a cellular membrane.

Oxidative phosphorylation is the process that allows the synthesis of ATP from the energy released during the transfer of electrons from membrane-bound electron carriers with the most negative redox potentials to those with more positive potentials (thermochemical principle: Nernst's Law).

The mechanism of oxidative phosphorylation has been the subject of intense study for years. The most widely accepted hypothesis for ATP production is the **chemiosmotic theory** (or **chemiosmotic coupling**), formulated by British biochemist Peter Mitchell in 1961.

According to this hypothesis, the electron carriers of the electron transport chain (such as various cytochromes, ubiquinone, iron/sulfur proteins, etc.) are organized (Fig. 10) so that, during the electron transport, protons are transferred across the membrane (extracellular space for bacteria), creating a **Proton Motive Force** (**PMF**). This force drives the return of protons into the cytoplasm, facilitating the synthesis of ATP at an enzyme located on the inner face of the cytoplasmic membrane, called ATP synthase (Fig. 11).

In bacteria, the electron transport chain components are located in the membrane (cytoplasmic membrane), and there are many variations. However, in eukaryotes, these components are located in the inner mitochondrial membrane.

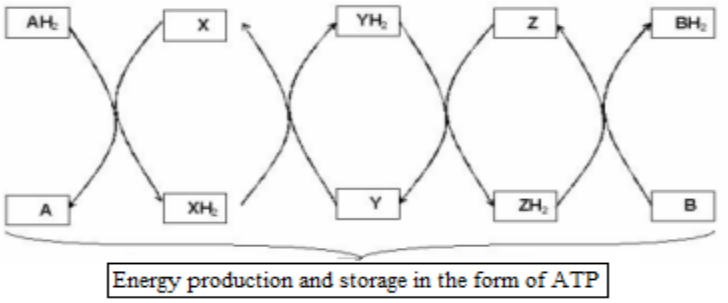

Figure 10: Schematic representation of the respiratory chain in bacteria

[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].

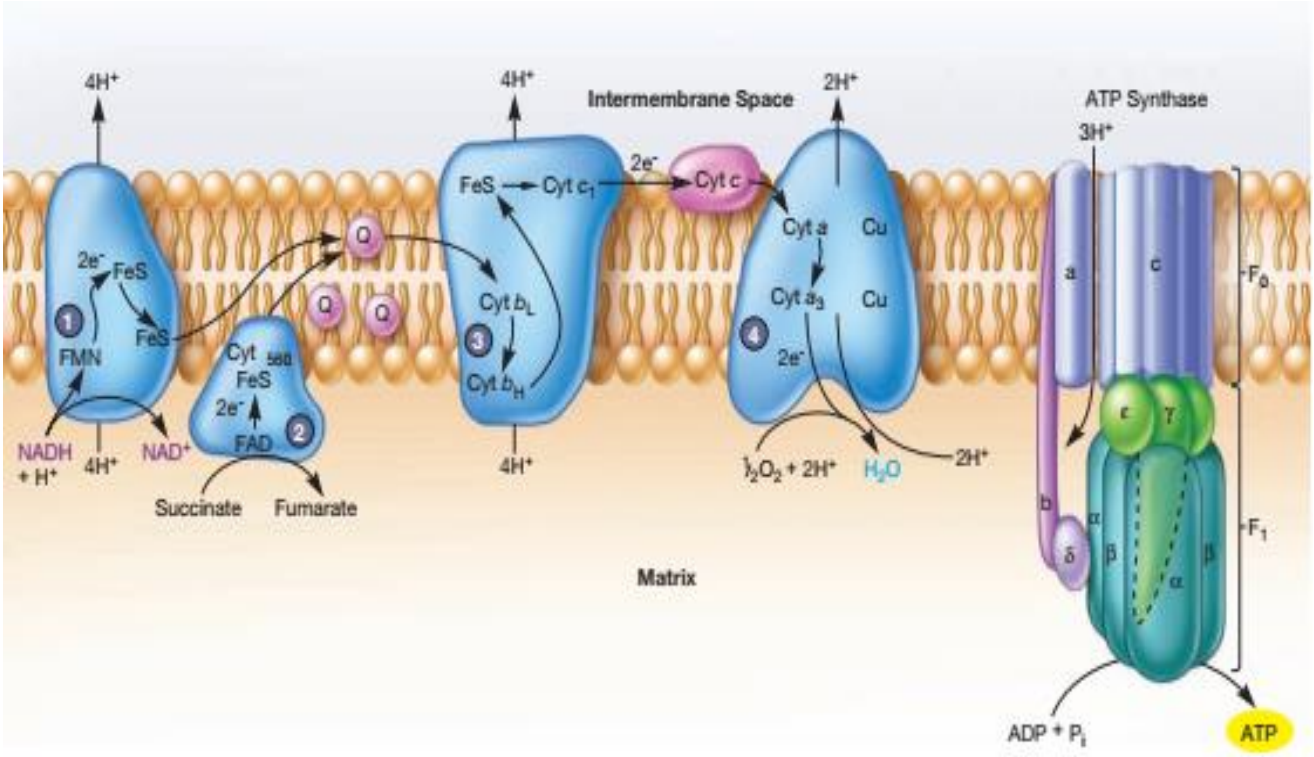

Figure 11: Chemiosmotic hypothesis applied to mitochondria.

[In this scheme the carriers are organized asymmetrically within the inner membrane so that protons are transported across as electrons move along the chain. Proton release into the intermembrane space occurs when electrons are transferred from carriers, such as FMN and coenzyme Q (Q), that carry both electrons and protons to components like nonheme iron proteins (FeS proteins) and cytochromes (Cyt) that transport only electrons. Complex IV pumps protons across the membrane as electrons pass from cytochrome *a* to oxygen. Coenzyme Q transports electrons from complexes I and II to complex III. Cytochrome *c* moves electrons between complexes III and IV. The number of protons moved across the membrane at each site per pair of electrons transported is still somewhat uncertain; the current consensus is that at least 10 protons must move outward during NADH oxidation. One molecule of ATP is synthesized and released from the enzyme ATP synthase for every three protons that cross the membrane by passing through it].

The more negative the electron potential of a system, the more energetic it is. The table below (Tab. 01) provides the standard potential for the main systems.

Table 01: Selected biologically important RedOx couples

Redox Couple	E'_0 (Volts) [a]
$2H^+ + 2e^- \rightarrow H_2$	−0.42
Ferredoxin (Fe^{3+}) + $e^- \rightarrow$ ferredoxin (Fe^{2+})	−0.42
$NAD(P)^+ + H^+ + 2e^- \rightarrow NAD(P)H$	−0.32
$S + 2H^+ + 2e^- \rightarrow H_2S$	−0.274
Acetaldehyde + $2H^+ + 2e^- \rightarrow$ ethanol	−0.197
$Pyruvate^- + 2H^+ + 2e^- \rightarrow lactate^{2-}$	−0.185
$FAD + 2H^+ + 2e^- \rightarrow FADH_2$	−0.18 [b]
$Oxaloacetate^{2-} + 2H^+ + 2e^- \rightarrow malate^{2-}$	−0.166
$Fumarate^{2-} + 2H^+ + 2e^- \rightarrow succinate^{2-}$	0.031
Cytochrome *b* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *b* (Fe^{2+})	0.075
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinone H_2	0.10
Cytochrome *c* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.421
$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$	0.44
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771 [c]
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	0.815

[a] *E*′0 is the standard reduction potential at pH 7.0.
[b] The value for $FAD/FADH_2$ applies to the free cofactor because it can vary considerably when bound to an apoenzyme.
[c] The value for free Fe, not Fe complexed with proteins (e.g., cytochromes).

In principle, the energy efficiency of long oxidative phosphorylation chains is higher than that of short chains. In bacteria, the presence of ATPases complicates the study of energy yields.

There are several classes of bacteria based on their relationship with oxygen, including:

- **Strict aerobes**: They only grow in the presence of air. Their primary source of energy is respiration. Molecular oxygen, the ultimate electron acceptor, is reduced to water (e.g., *Pseudomonas, Acinetobacter, Neisseria*).

• **Microaerophiles**: They grow better or exclusively when the partial pressure of oxygen is lower than that of air (*Campylobacter*, *Mycobacteriaceae*).

• **Facultative aero-anaerobes**: They can grow with or without air, including enterobacteria (*Escherichia*, *Salmonella*), *Streptococcus*, and *Staphylococcus*. Energy comes from substrate oxidation and fermentation pathways.

• **Strict anaerobes**: They only grow in the complete or near-complete absence of oxygen, which is often toxic. These bacteria must grow under a reducing atmosphere. All of their energy is produced by fermentation. This includes intestinal bacteria (*Bacteroides*, *Fusobacterium*, *Clostridium*) and many bacteria in normal flora. Energy production occurs via membrane cytochromes coupled with oxidative phosphorylation, but in the absence of molecular oxygen. Oxygen's toxicity is due to the production of superoxide radicals, which anaerobic bacteria cannot destroy (lack of superoxide dismutase) and/or the absence of enzymes such as catalases and peroxidases.

2.2. Anaerobic respiration

This is a process where the final electron acceptor is an oxidized mineral substance (an exogenous terminal electron acceptor). Many microorganisms are capable of completely oxidizing glucose in the absence of air, provided that nitrate is present in the medium. In addition to nitrates, other substances can be used: nitrites, sulfates, sulfur, CO_2, etc.

During anaerobic respiration, the degradation of the organic hydrogen source (H^+, e^-) may not be complete and may result in other substances (organic acids).

Table 02: Different electron acceptors used in anaerobic respiration in bacteria

Electron acceptor	Reduced product	Process name	Example of Microorganisms
NO_3^-	NO_2^-, N_2O, or N_2	Anaerobic respiration (Denitrification)	*Bacillus, Pseudomonas*
SO_4^{2-}	H_2S	Anaerobic respiration (Sulfate reduction)	*Desulfovibrio*
Fumarate	Succinate	Anaerobic respiration using an organic electron acceptor	*Escherichia coli*
CO_2	CH_4	Methanogenesis	*Methanococcus*
S0	H_2S	Anaerobic respiration	*Desulfuromonas*

Some anaerobic bacteria (and strict anaerobes) do not require oxygen, and it is often toxic. Oxygen can chemically produce superoxide ions (O_2^-), hydroxyl radicals (HO.), and hydrogen peroxide (H_2O_2),

which can oxidize bacterial cell lipids and proteins. These bacteria lack protective enzymes such as superoxide dismutase, catalase, or peroxidase.

2.3. Fermentation

In fermentation, the final electron (and proton) acceptor is an organic molecule, usually endogenous, and the transfer of electrons from the energy substrate (electron donor) occurs without passing through a membrane transport chain, but by simple coupling between the initial oxidation reaction and a phosphorylation reaction: "**Substrate-Level Phosphorylation**."

Fermentation leads to the accumulation of reduced organic molecules, which are generally eliminated as waste in the culture medium. This part of the energetic metabolism is very complex because the metabolic pathways and biochemical reactions are numerous and varied.

Many fermentations can occur under anaerobic conditions, as all electrons and protons from substrate oxidation are used to reduce the organic acceptor (as in homolactic fermentation). For other fermentations, only part of the electrons and protons is used: oxygen acts as a complementary acceptor, either facultatively (certain bacterial heterolactic fermentations) or obligatorily (fermentation of pentoses by certain yeasts).

Fermentation is used by:

- Facultative aero-anaerobic bacteria, which preferentially use respiration when possible;
- Strict anaerobic bacteria (e.g., *Clostridium*).

Three unifying themes must be kept in mind when studying microbial fermentations:

- NADH is oxidized to NAD+;
- The electron acceptor is often either pyruvate or a pyruvate derivative;
- Oxidative phosphorylation cannot function, which significantly reduces ATP yield. In fermentation, the substrate is only partially oxidized.

The energy yield of fermentations is lower than that of respirations:

aerobic respiration > anaerobic respiration > fermentation.

2.4. Energy impact of aerobiosis and anaerobiosis

"Classical" respiratory metabolism results in the complete degradation of the substrate, thus releasing a significant amount of electrons (and protons). In contrast, fermentation corresponds to

incomplete oxidation, leading to a lesser release of electrons. The number of electron and proton pairs released, the nature of the redox chain, and the nature of the acceptor condition the energy yield by the number of ATP formed.

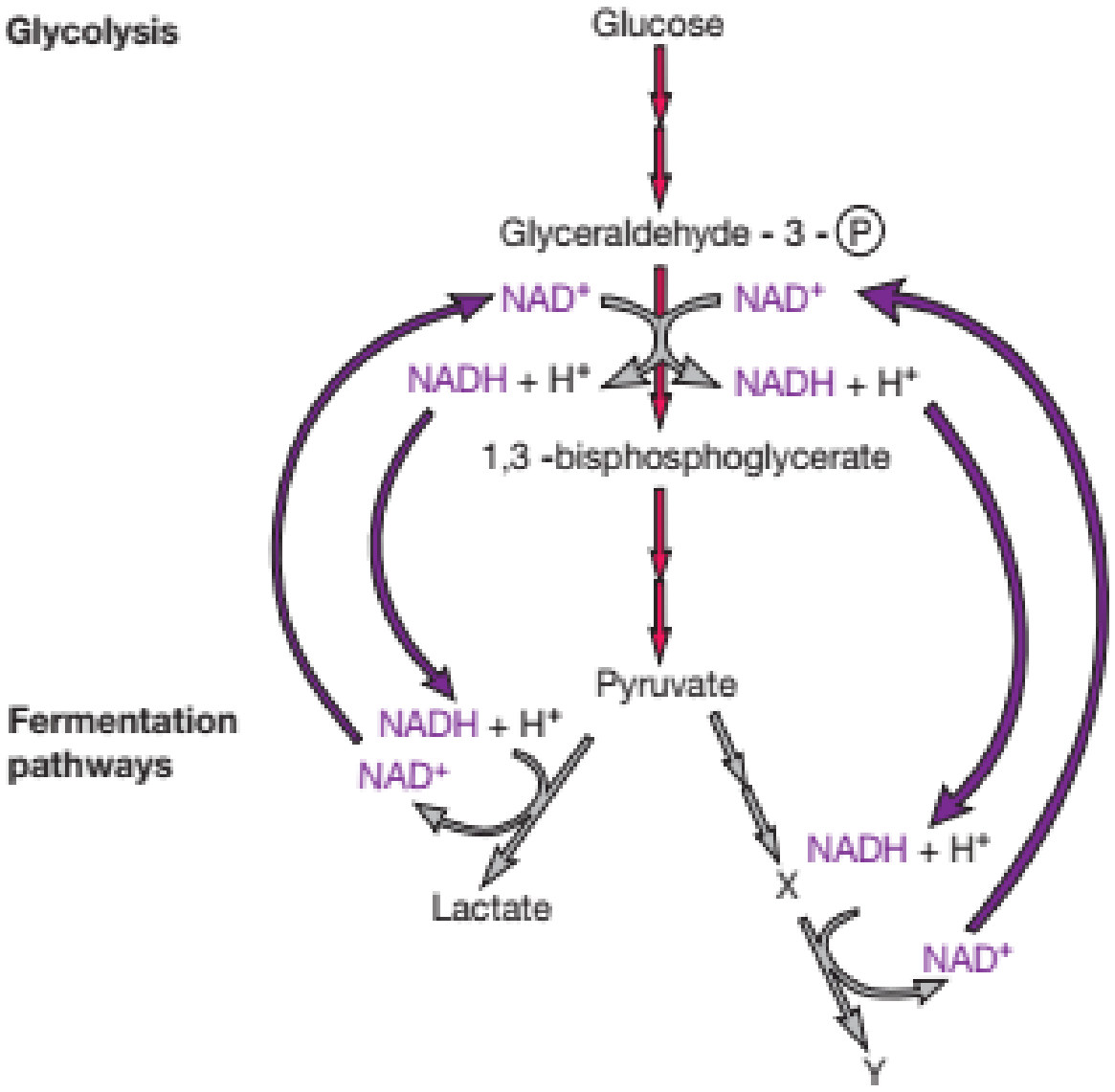

Figure 13: Reoxidation of NADH during fermentation

[NADH from glycolysis is reoxidized by being used to reduce pyruvate or a pyruvate derivative (X). Either lactate or reduced product Y result].

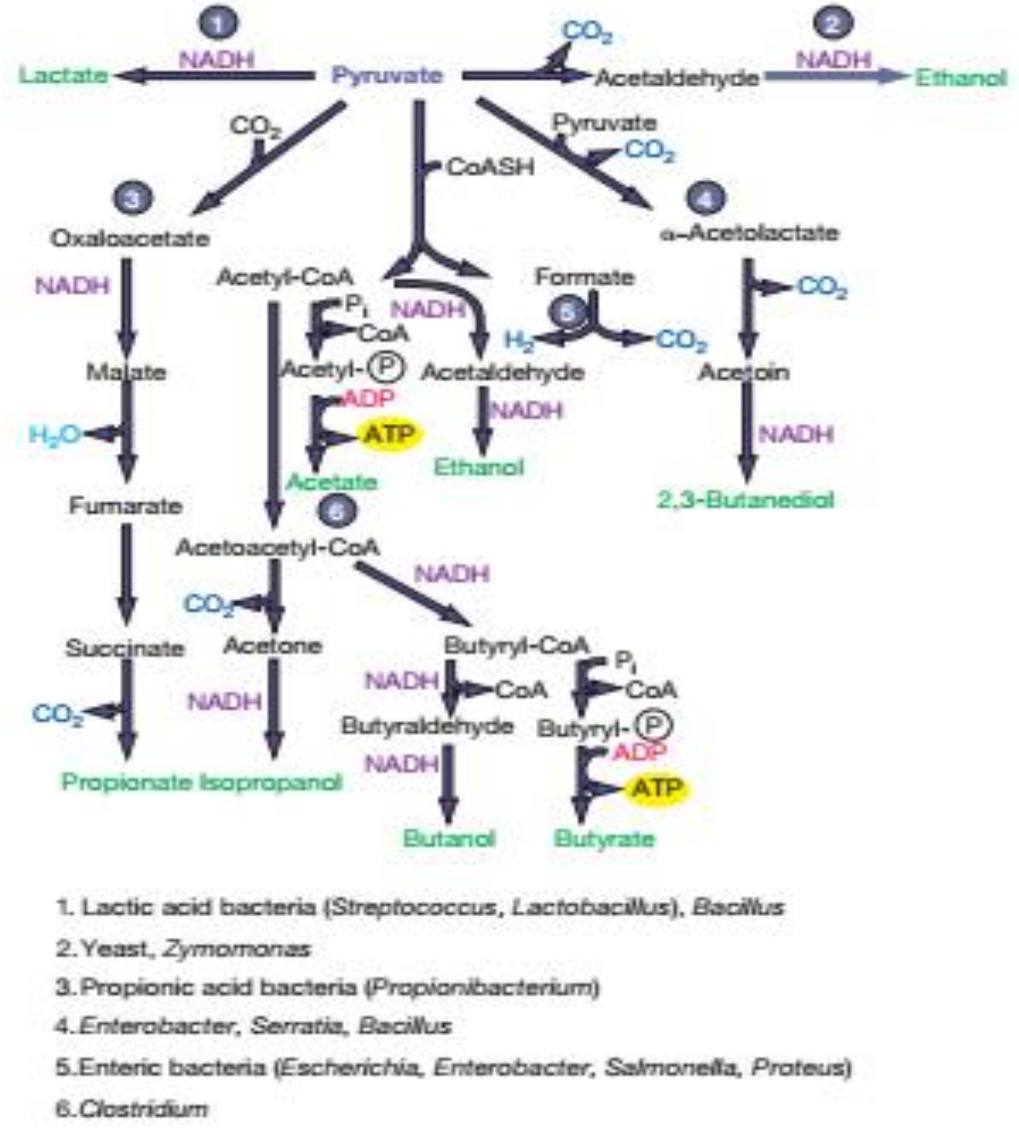

Figure 14: Some common microbial fermentations

[Only pyruvate fermentations are shown for the sake of simplicity; many other organic molecules can be fermented. Most of these pathways have been simplified by deletion of one or more steps and intermediates. Pyruvate and major end products are shown in color].