# Microbial Metabolism

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# BACTERIAL METABOLISM

- **METABOLISM** = the series of changes of a substance (carbohydrate, protein, fat) that take place within the bacterial cell from absorption to elimination is known as metabolism.

- **CATABOLISM** = breakdown of macromolecules into simpler micromolecules, absorption into cell, conversion into basic blocks including interconversion of ADP to ATP.

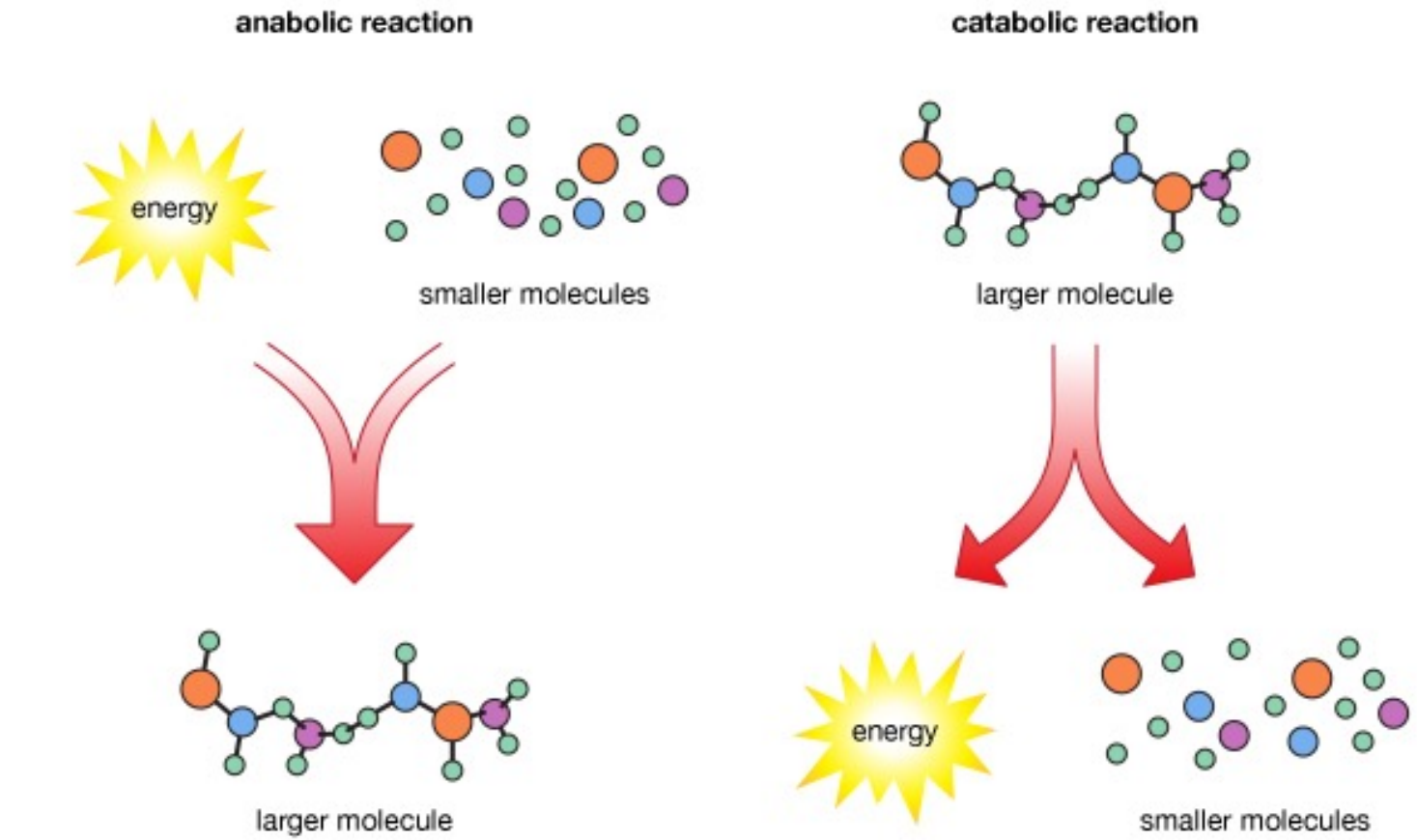
- **ANABOLISM** = a process by which the basic building blocks are utilized in synthesis of various cellular structures such as monomers and polymers.

- Aerobic bacteria obtain their energy and intermediates only through **OXIDATION** and energy is provided by ATP (oxidative phosphorylation).

- Anaerobic bacteria obtain their energy by **FERMENTATION** (substrate level phosphorylation).

- Facultative anaerobes may act in both ways.

## Metabolism

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- Glucose is a key energy-storing molecule
- Nearly all cells metabolize glucose for energy
- Glucose metabolism is fairly simple
- Other organic molecules are converted to glucose for energy harvesting

# Components of metabolism

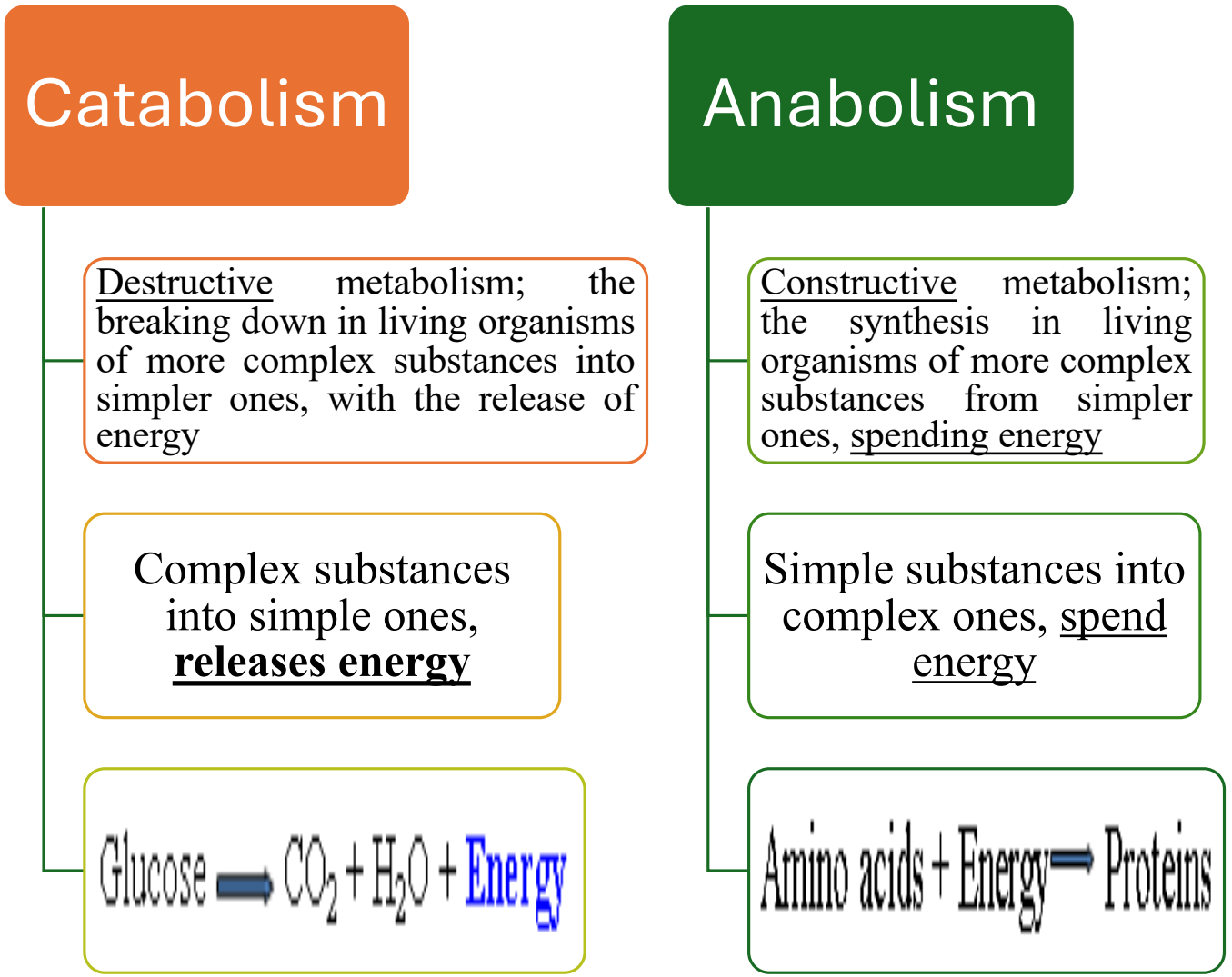
| Components | Functions |
|---|---|
| Enzymes | biological catalyst, facilitates each step of metabolic reaction by lowering the activation of energy reaction. |
| Adenosine triphosphate (ATP) | Serves as energy currency of cell |
| Energy source | Compound that is oxidized to release energy, also called as electron donor. |
| Electron carriers | Carry the electrons that are removed during oxidation of energy source. |
| Precursor metabolites | Intermediate metabolite that link anabolic & catabolic pathway. |

# MICROBIAL METABOLISM

The sum of all chemical reactions within a living organism

Catabolism

Anabolism


Catabolism
Destructive metabolism; the breaking down in living organisms of more complex substances into simpler ones, with the release of energy
Complex substances into simple ones, releases energy
Glucose ⟶ $CO_2 + H_2O$ + Energy
Anabolism
Constructive metabolism; the synthesis in living organisms of more complex substances from simpler ones, spending energy
Simple substances into complex ones, spend energy
Amino acids + Energy ⟶ Proteins

# Chemical principles of metabolism

A part of the energy released from oxidation of foodstuffs and from light gets stored in a molecule, is known as **adenosine triphosphate (ATP).**
It, then, transfers this energy to reactions that require energy.
ATP functions as a carrier of energy in all living organisms including bacteria, fungi, plants and animals.

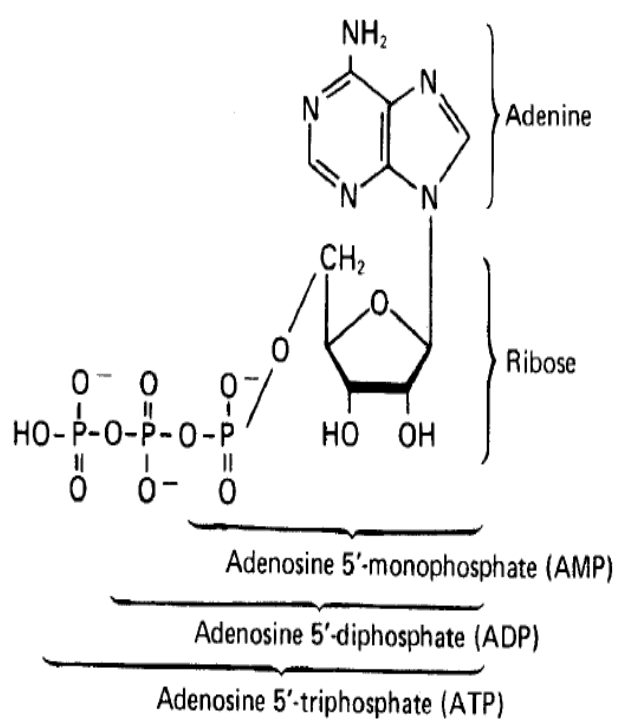
**ATP hydrolysis:**

$$ATP + H_2O \underset{\text{Catabolism}}{\overset{\text{Anabolism}}{\rightleftharpoons}} ADP + Pi \; [\Delta G^0 = -30.5 \text{ KJ/mol}]$$

The amount of energy in ATP is written in terms of standard free energy change, $\Delta G^0$.

ATP was discovered in **1929** by **Karl Lohmann**. It was proposed to be the main energy-transfer molecules in the cell by **Fritz Albert Lipmann** in **1941**.

ATP is a nucleotide consisting of apurine **base** (adenine), a pentose **sugar** (ribose) and three **phosphate** groups (triphosphate unit). Adenine is attached to the 1'carbon atom and the phosphate groups are attached at the 5'carbon atom of the ribose. ATP, in its active form, exists as a complex of ATP with $Mg^{2+}$ or $Mn^{2+}$ ions.




**The structure of adenosine triphosphate (ATP)**

ATP is a derivative of AMP to which two additional phosphates groups are attached through an **anhydrous linkage** (high energy bonds, ~). And thus are particularly reactive. Hence ATP is able to donate phosphate groups to a number of metabolic intermediates, thereby converting them to activated forms.

Adenosine—P~P~P + $H_2O$ → Adenosine—P~P + P $\Delta G^0$ = -30.5 Kcal

Adenosine—P~P + $H_2O$ → Adenosine—P + P $\Delta G^0$ = -30.5 Kcal

In AMP where adenosine is attached to phosphate by an **ester linkage**, therefore less free active and termed a low energy bond (—).

Adenosine—P~P~P + $H_2O$ → Adenosine—P + PPi $\Delta G^0$ = -40.6 Kcal

PPi → 2Pi $\Delta G^0$ = -31.8 Kcal

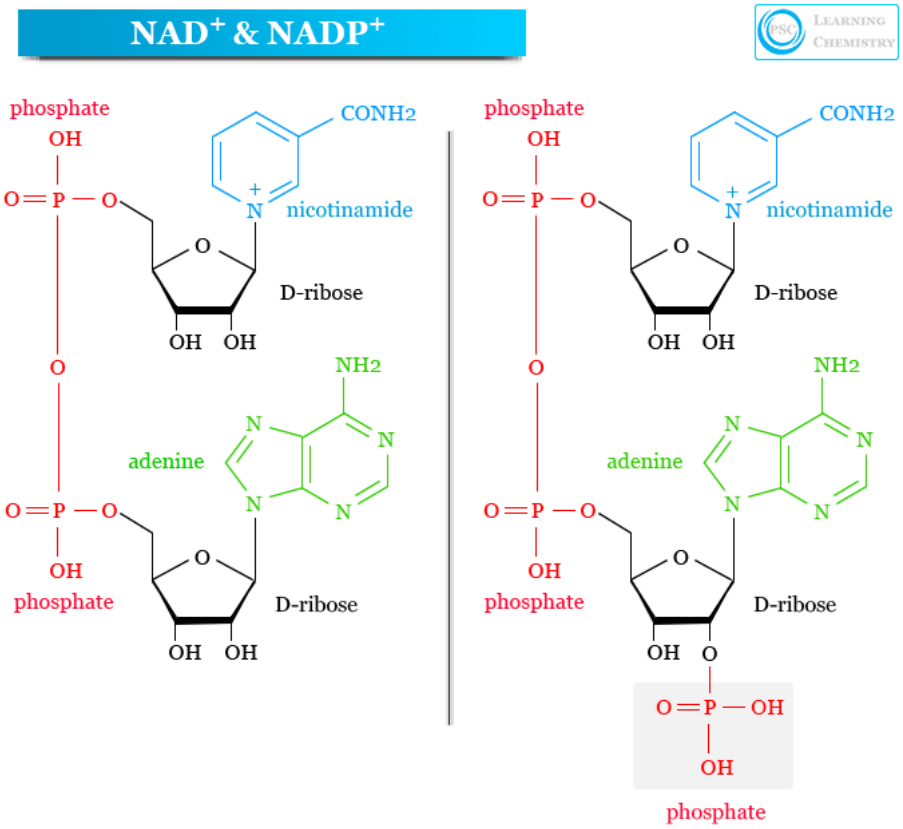
The compound that mediate biological oxidation and reductions are two pyridine nucleotides:

**Nicotinamide adenine dinucleotide (NAD)**

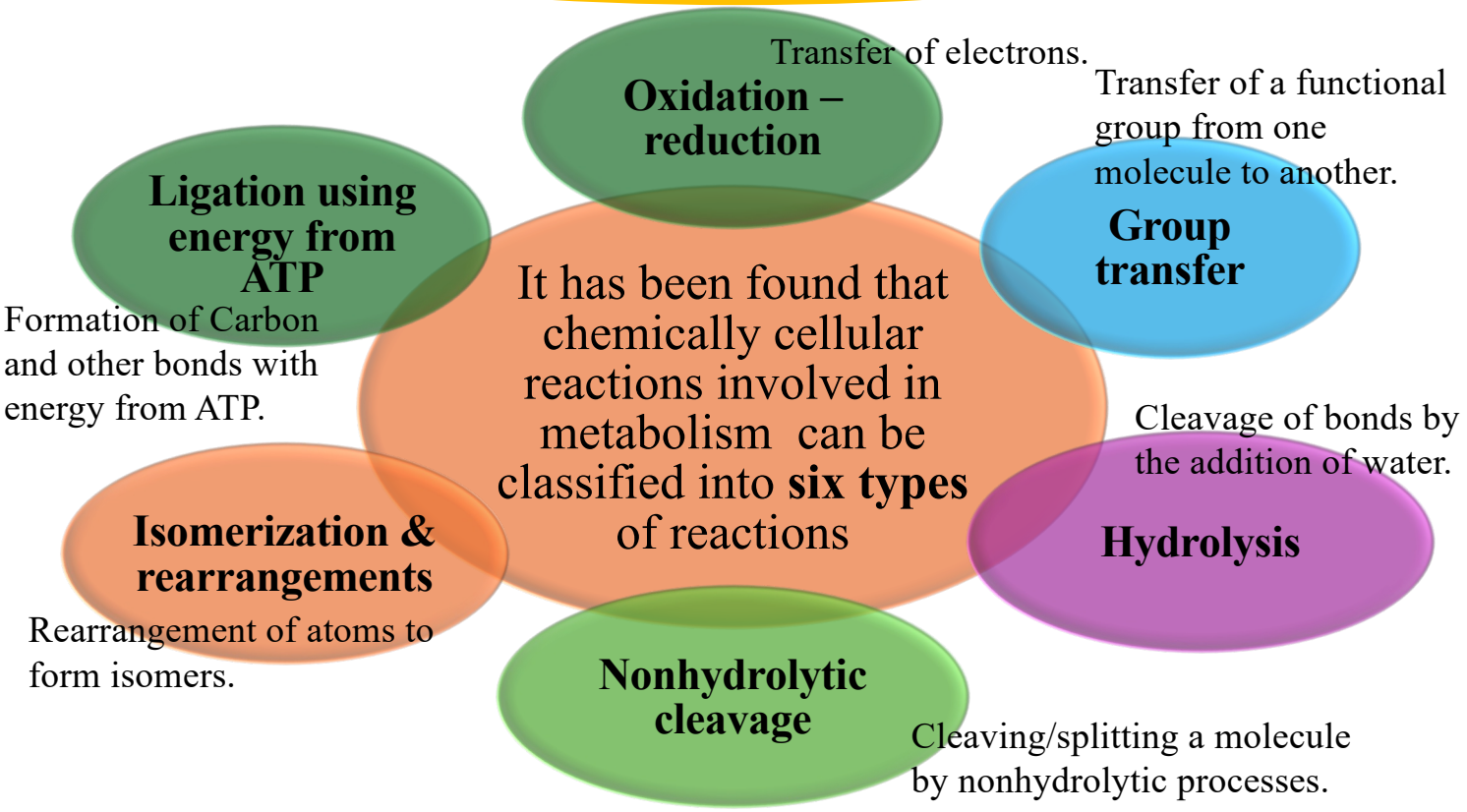
**Nicotinamide adenine dinucleotide phosphate (NADP)**

Both of these pyridine nucleotides can **undergo reversible oxidation** and **reduction**.

In cataboilc reactions oxidized pyridine nucleotides are the usual reactant, and in biosynthetic reactions the reduced form is the usual reactant. Consequently, **two kinds** of pyridine nucleotides **are required**. Indeed, the intracellular pool of NAD is maintained largely in the oxidized state, and the pool of NADP is maintained largely in the reduced state.



# *Chemistry of metabolism*

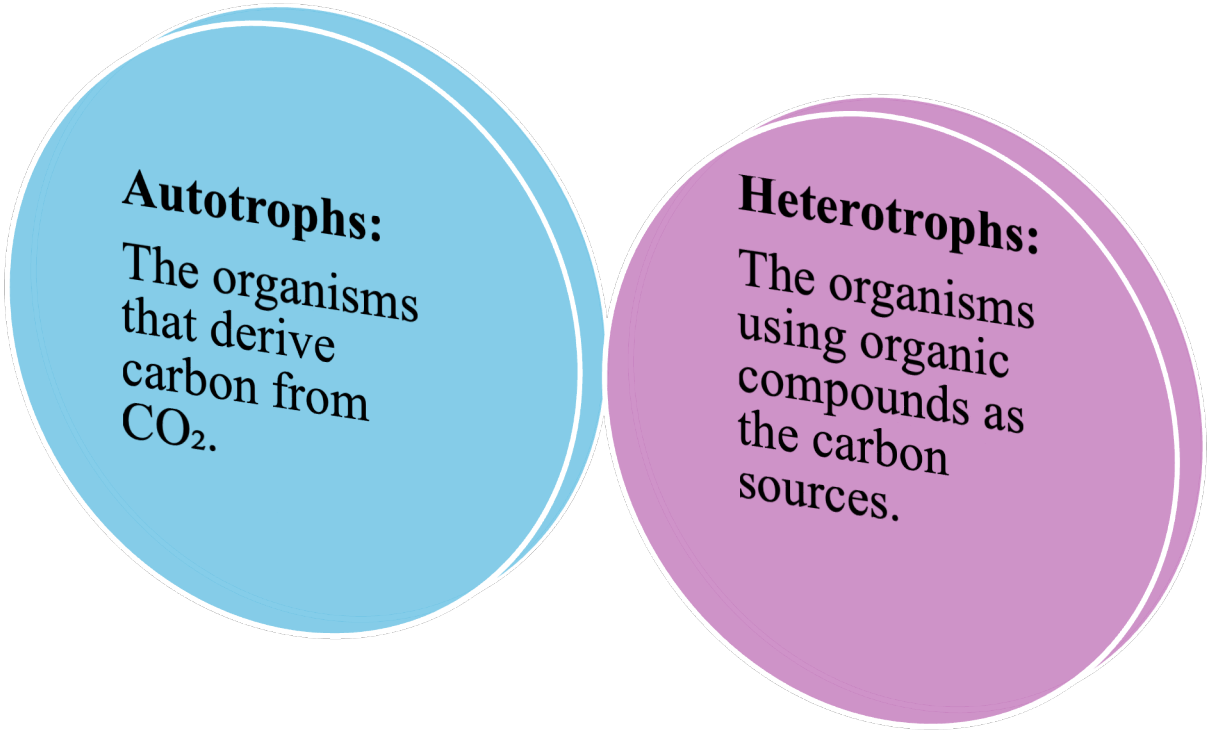


# Metabolic diversity in microbial world

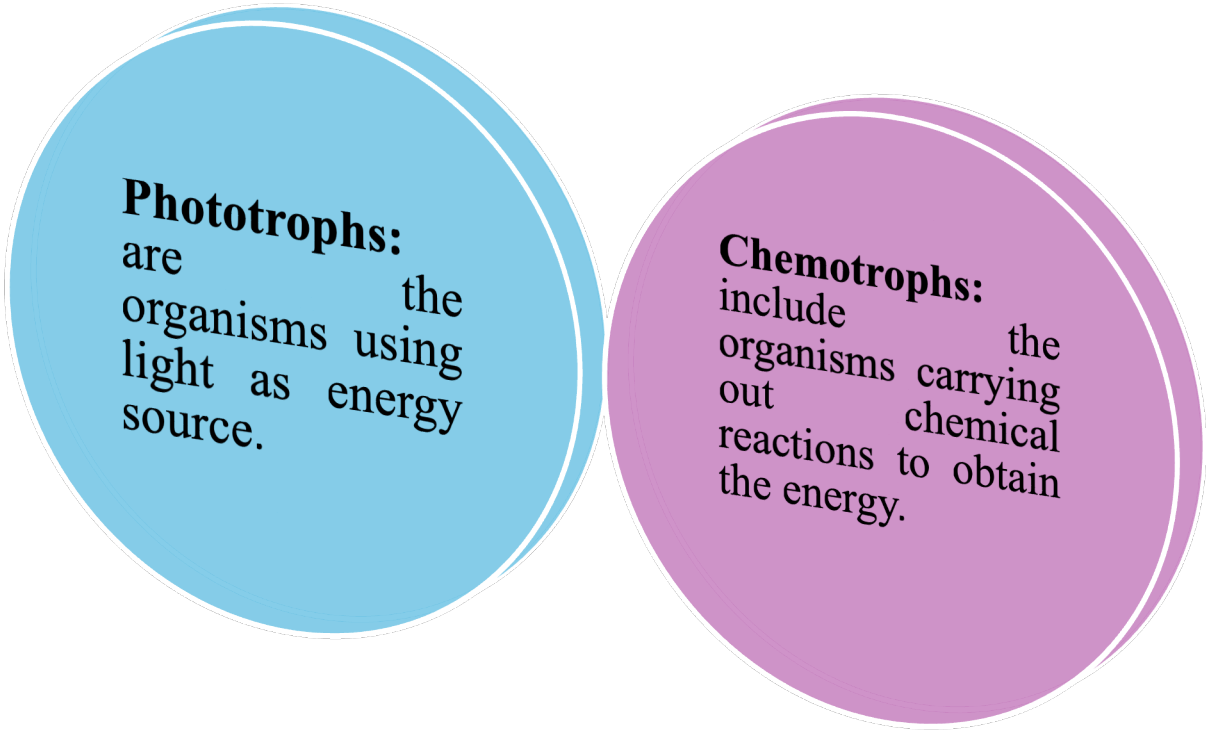
1. Green plants are photosynthetic. They obtain their energy from sunlight and use $CO_2$ as their carbon source.
2. Animals require preformed organic molecules for energy as well as building blocks.

The nutritional needs of microorganisms vary from species to species. Depending on the environmental conditions, they can utilize a diverse range of substrates as carbon or energy sources e.g. sugars, amino acids, CO, methane, cyanide, acetic acid, $CO_2$ etc.

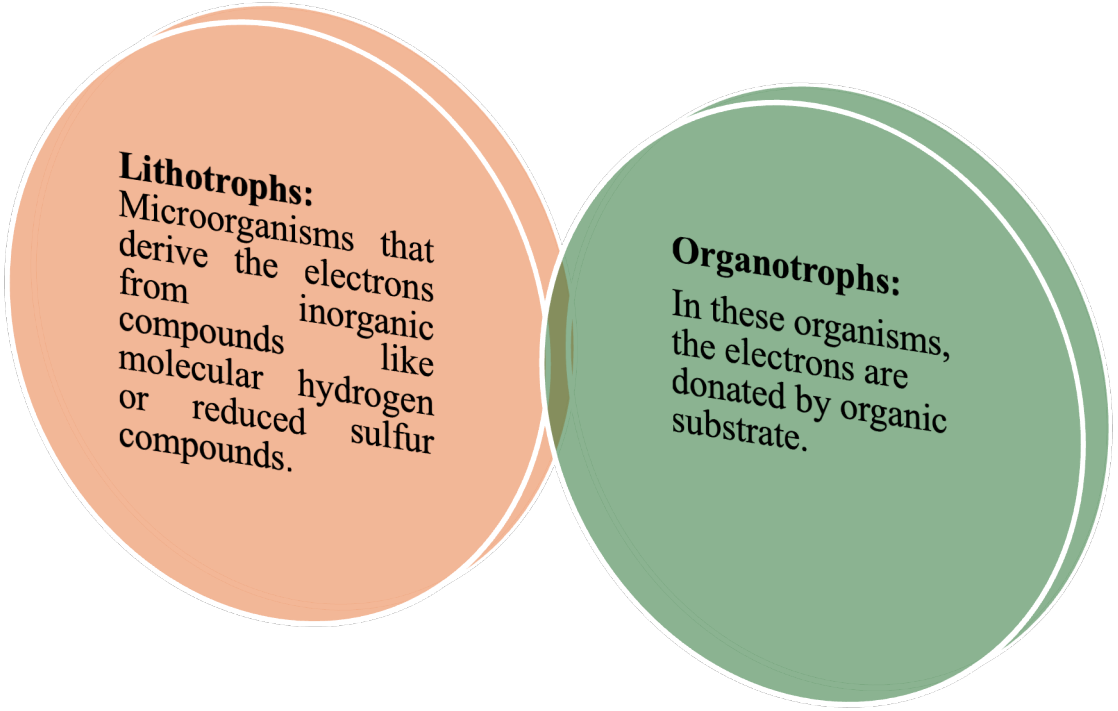
The microbes can be divided into two groups based on their carbon sources:



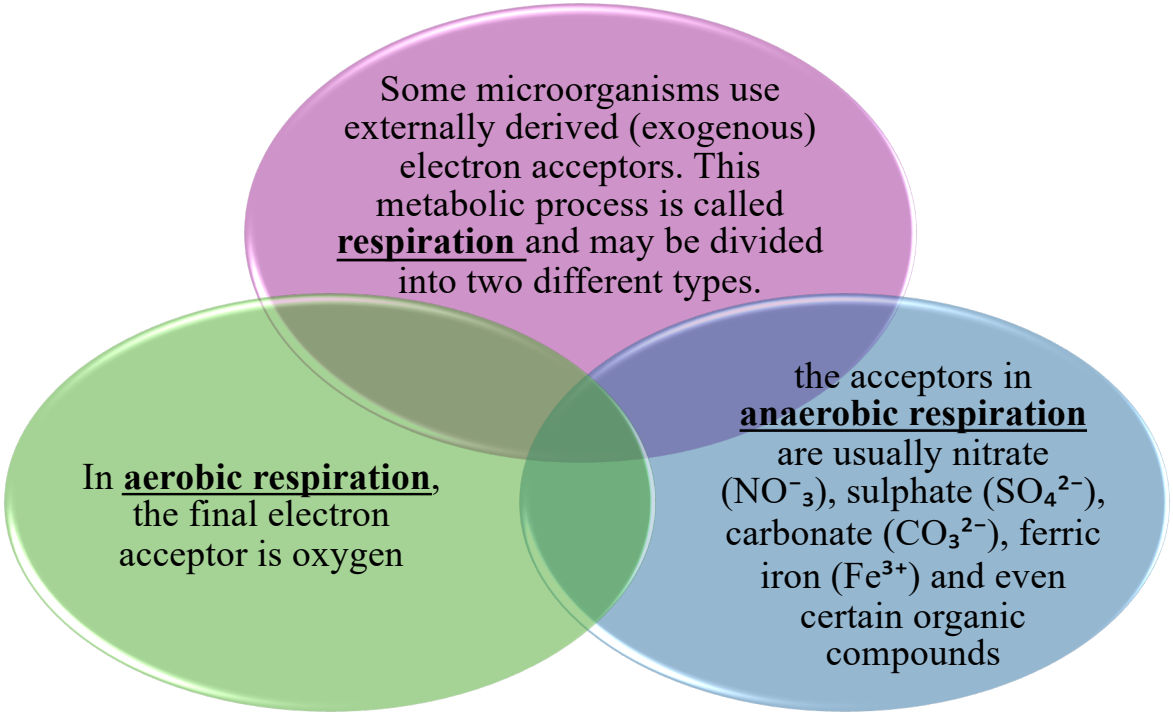
On the basis of energy sources, microbes are of two types:



Besides carbon and energy sources, microorganisms also require a source of electrons for the reduction reactions.



During metabolism, electrons are also released. Hence, electron acceptors are also required.




The ATP in most respirations is formed as a result of the activity of an electron transport chain.

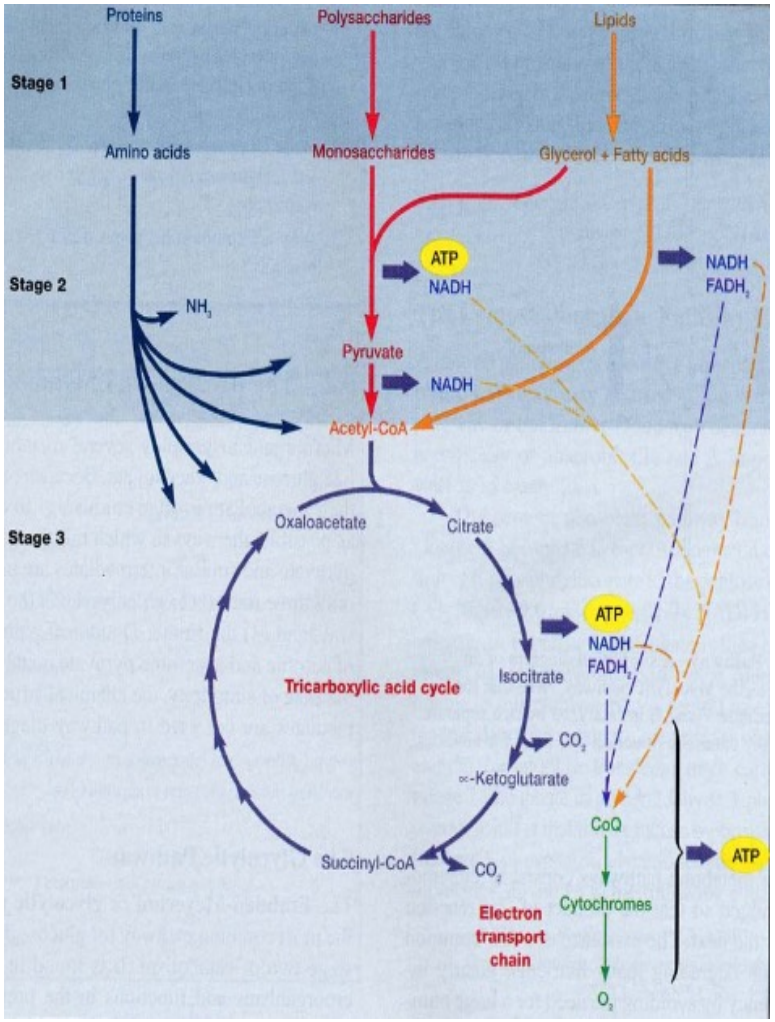
Sometimes, under anaerobic conditions the substrate is oxidized and degraded without the participation of an external electron acceptor. Usually, the catabolic pathway produces an intermediate such as pyruvate that acts as the electron acceptor. Such processes where an internally derived (endogenous) organic electron acceptor is used, is called as **fermentation**.

# Chemoorganoheterotrophs

**Phase I - Breakdown of large complex** biomolecules like polysaccharides, proteins and lipids **into** their respective **building blocks**. The chemical reactions occurring during this stage do not release much energy.

**Phase II** - These building blocks are usually **oxidized** to a common intermediate, **acetyl - CoA**. In addition, pyruvate or other citric acid cycle intermediates may also be formed.

**Phase III** - It consists of the **citric acid cycle** (i.e. oxidation of acetyl - CoA to CO2, formation of NADH and FADH2) followed by **electron transport** and **oxidative phosphorylation**. Energy released by electron transport of O2 is coupled to ATP synthesis. This cycle is responsible for the release of much energy.



# Overview of Glucose Breakdown

- The overall equation for the complete breakdown of glucose is:


- The main stages of glucose metabolism are:
  - **Glycolysis**
  - **Cellular respiration**

Most of the microbes can grow on a variety of polysaccharides. However, these polysaccharides are too large to be taken up by the cells.

Therefore, in phase I microorganisms excrete exoenzymes that hydrolyse these large molecules into small transportable sugar molecules.

These molecules are then degraded to pyruvate and similar intermediates in Phase II by any of the 3 routes:

(i) Glycolysis
(ii) The pentose phosphate pathway and
(iii) The Enter- Doudoroff pathway.

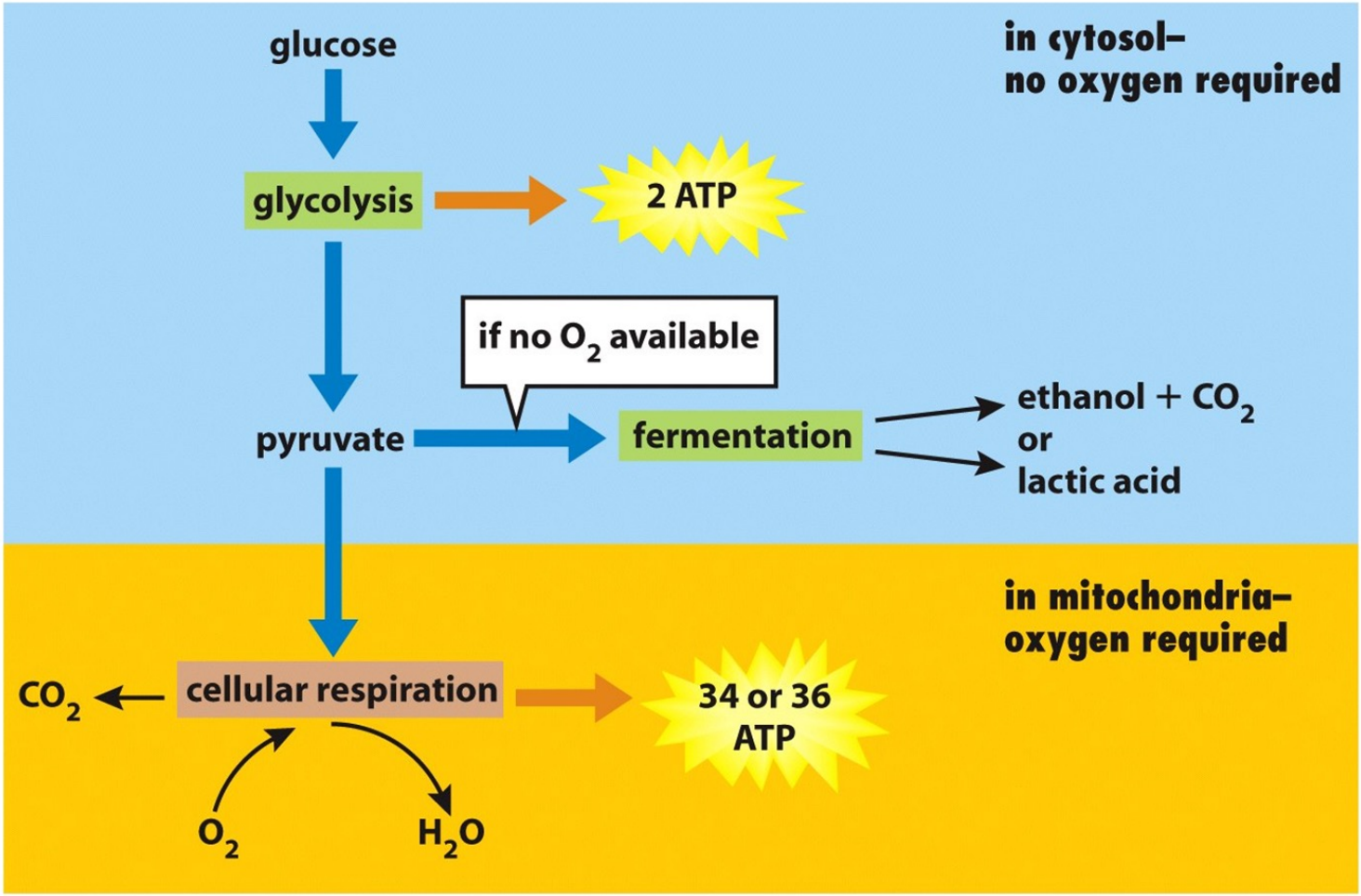



Figure 8-1 Biology: Life on Earth, 8/e

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# Overview of Glucose Breakdown - Glycolysis

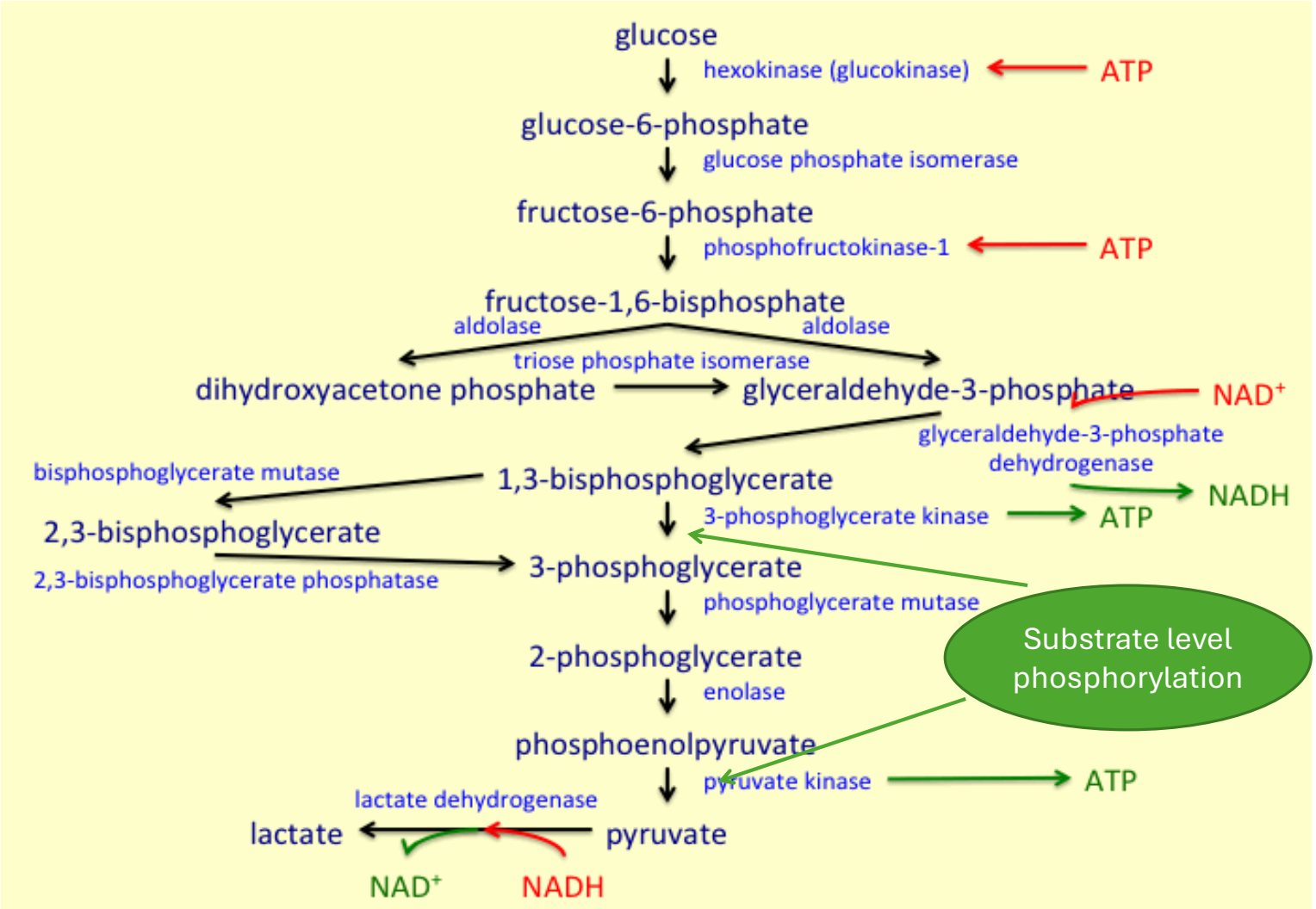
- Glycolysis
- Occurs in the cytosol
- Does not require oxygen
- Breaks glucose into pyruvate
- Yields two molecules of ATP per molecule of glucose

# The Glycolytic Pathway

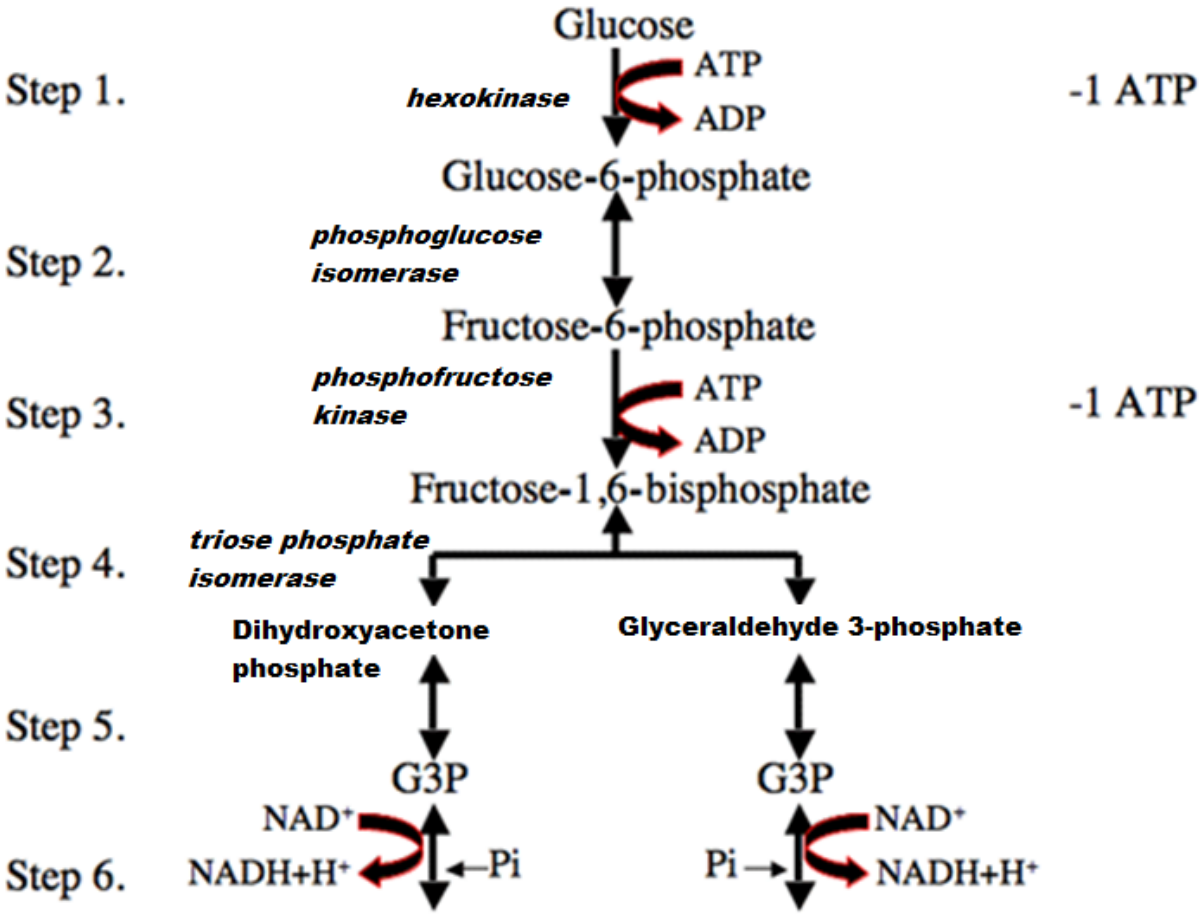
The pathway is also known as Embden – Meyerhof – Parnas (EMP) pathway. It is the most common pathway for glucose degradation to pyruvate and is found in animals, plants and large number of microorganism. This pathway is used by anaerobic as well as aerobic organisms. The process takes place in the cytoplasm of prokaryotes and eukaryotes.

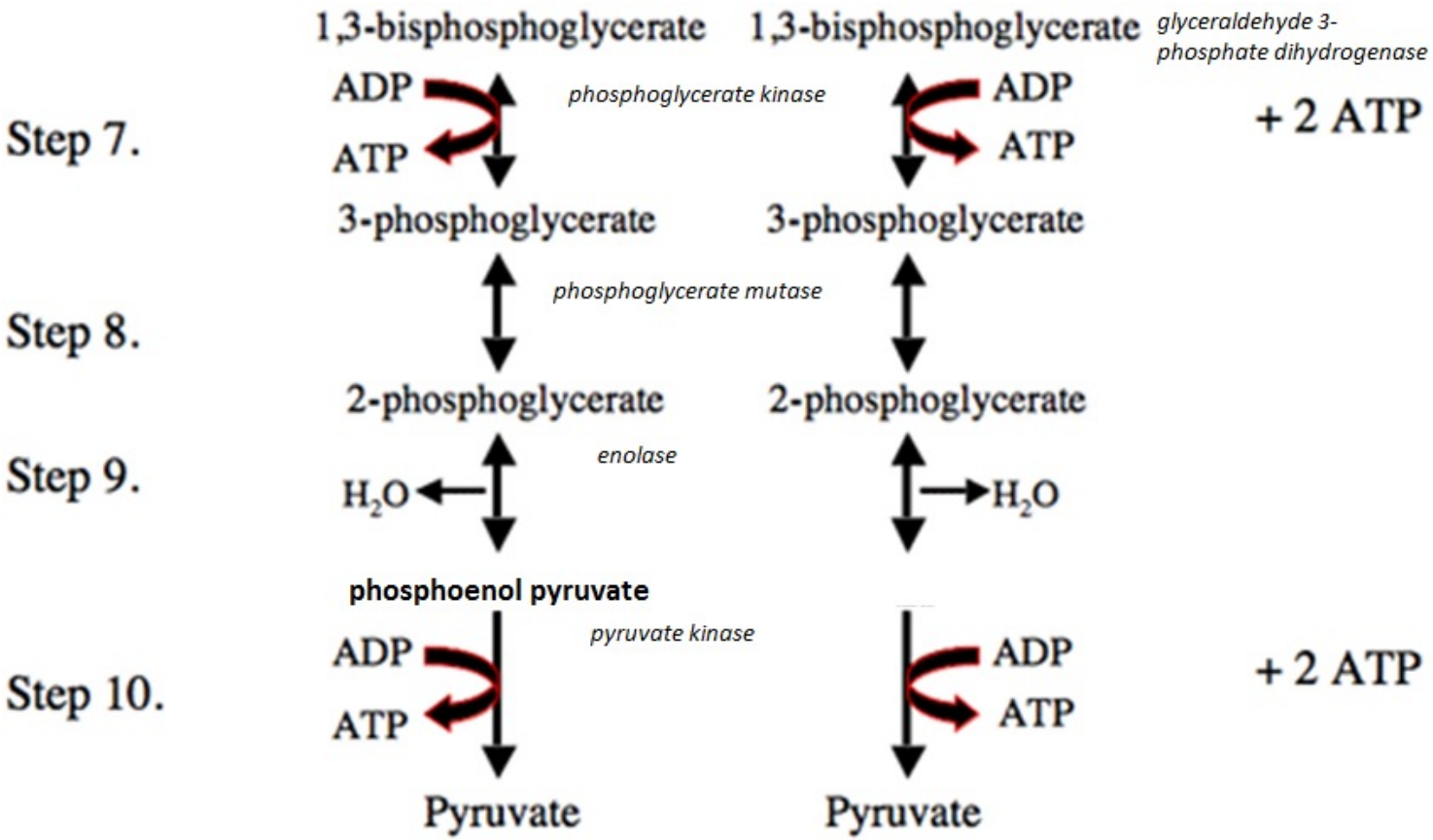
The pathway consists of ten enzyme-catalyzed reactions that begin with a glucose molecule. These reactions comprise three stages:

(i) Conversion of glucose into fructose 1,6 - bisphosphate
(ii) Splitting of the fructose 1-6- bisphosphate into two three-carbon fragments.
(iii) The formation of pyruvate along with ATP generation.




$$\text{Glucose} + 2_{P_i} + 2\ \text{ADP} + 2\text{NAD}^+ \longrightarrow 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\text{NADH} + 2\text{H}^+ + 2\text{H}_2\text{O}$$


Glucose
Step 1.
hexokinase
ATP
ADP
-1 ATP
Glucose-6-phosphate
Step 2.
phosphoglucose isomerase
Fructose-6-phosphate
Step 3.
phosphofructose kinase
ATP
ADP
-1 ATP
Fructose-1,6-bisphosphate
Step 4.
triose phosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Step 5.
G3P
G3P
Step 6.
NAD+
NADH+H+
Pi
Pi
NAD+
NADH+H+


1,3-bisphosphoglycerate
1,3-bisphosphoglycerate
glyceraldehyde 3-phosphate dihydrogenase
Step 7.
ADP
ATP
phosphoglycerate kinase
ADP
ATP
+ 2 ATP
3-phosphoglycerate
3-phosphoglycerate
Step 8.
phosphoglycerate mutase
2-phosphoglycerate
2-phosphoglycerate
Step 9.
enolase
H2O
H2O
phosphoenol pyruvate
Step 10.
ADP
ATP
pyruvate kinase
ADP
ATP
+ 2 ATP
Pyruvate
Pyruvate

- If oxygen is absent fermentation occurs
- **Pyruvate is converted into either lactate, or into ethanol and $CO_2$**
- If oxygen is present cellular respiration occurs

# Alternative Pathways

- Many bacteria have another pathway in addition to glycolysis for degradation of glucose.

**1. Pentose Phosphate Pathway**

**2. Entner Doudoroff Pathway**

# Pentose phosphate pathway

Most of the catabolic pathways generate NADH as the reducing agent (the source of electrons). However, many anabolic pathways require NADPH as reducing agent.

For this microorganisms may use a secondary pathway, the pentose phosphate or hexose monophosphate pathway, along with glycolysis or ED pathway.

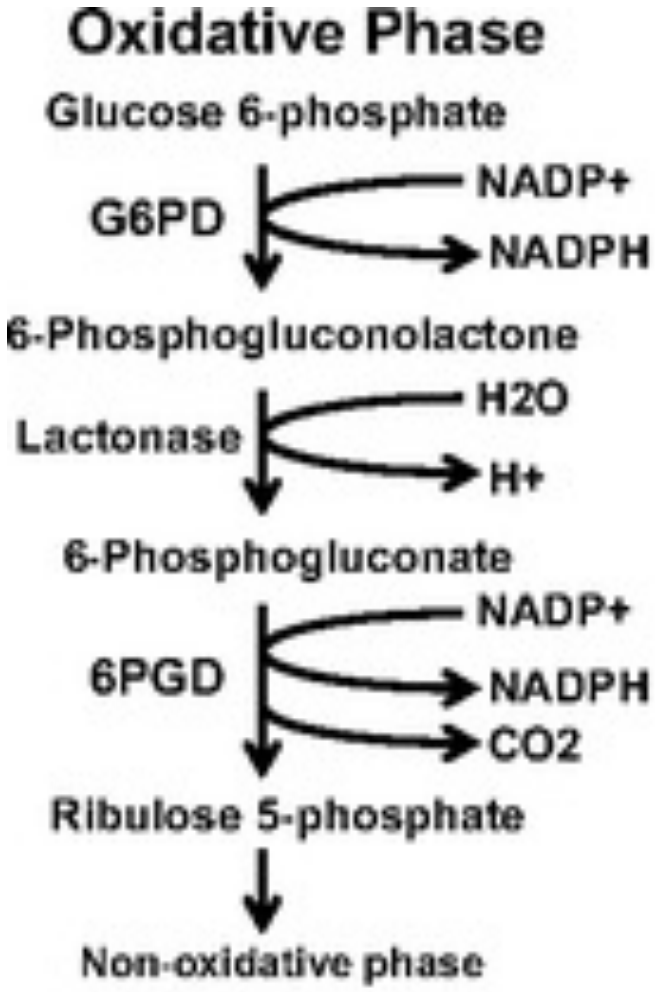
It can take place in aerobic or anaerobic conditions and is important in biosynthesis as well catabolism.

# Pentose phosphate pathway

- Hexose monophosphate shunt
- Occurs simultaneously with glycolysis & provide breakdown of both pentose sugar and glucose.
- Intermediate pentoses are used for nucleic acid synthesis, amino acid synthesis
- Important producer of reduced coenzyme i.e. NADPH used for biosynthetic reaction.

*Stuart Hogg Essential microbiology 2nd edition John Wiley & Sons.Ltd 2005*

The pathway begins with the oxidation of G-6-P to 6-phosphogluconate followed by the oxidation of 6-phosphogluconate to the ribulose- 5-P and CO2. NADPH is produced during these oxidations. Ribulose 5- P is then converted to a mixture of sugar phosphates (varying from 3 carbon to 7 carbon).



The enzymes carrying out these reactions are:

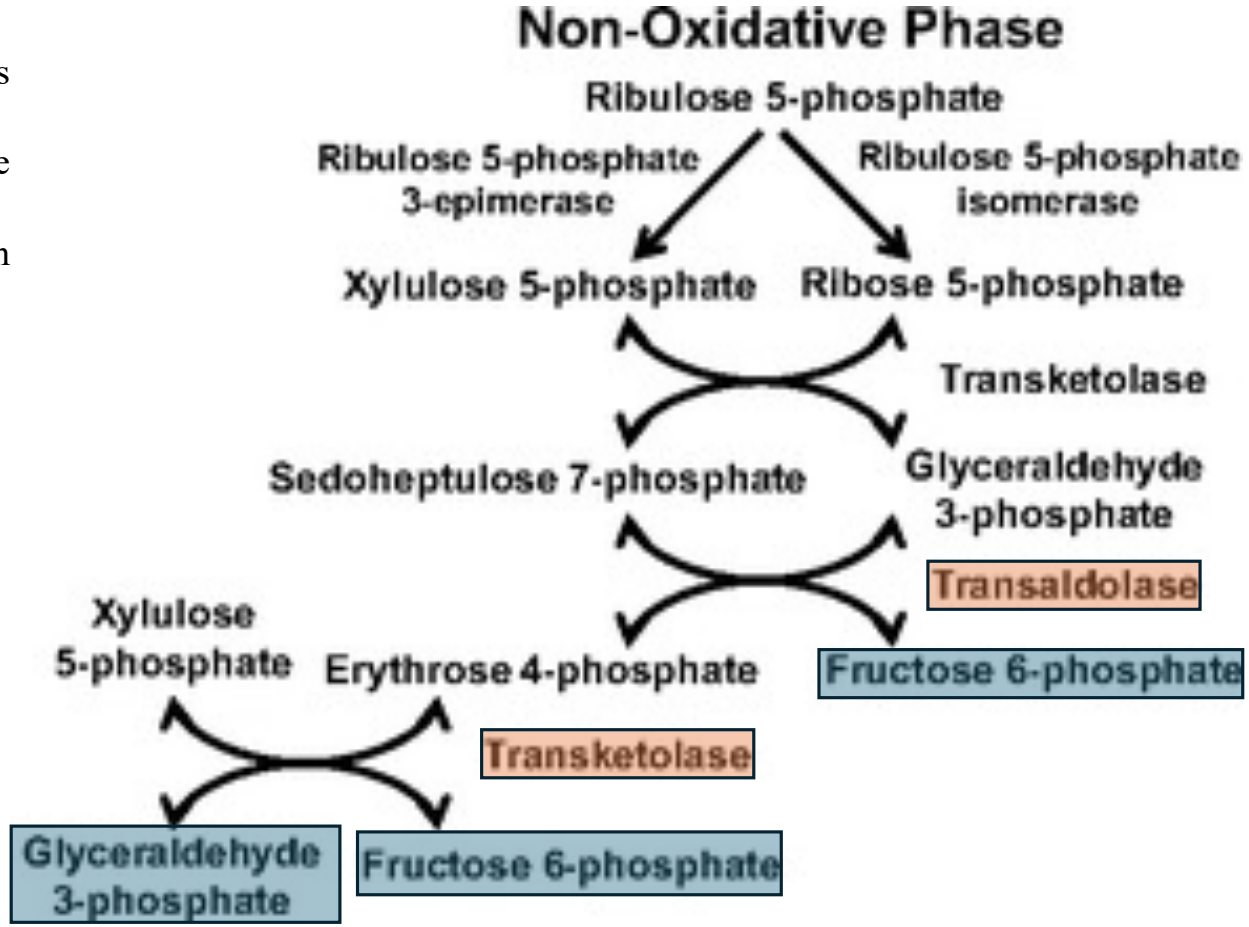
**(i)** **Transketolase**. It catalyzes the transfer of 2-carbon keto groups.

**(ii)** **Transaldolase**. It transfers a 3-carbon group from

sedoheptulose 7-phosphate

to

glyceraldehyde - 3-P.



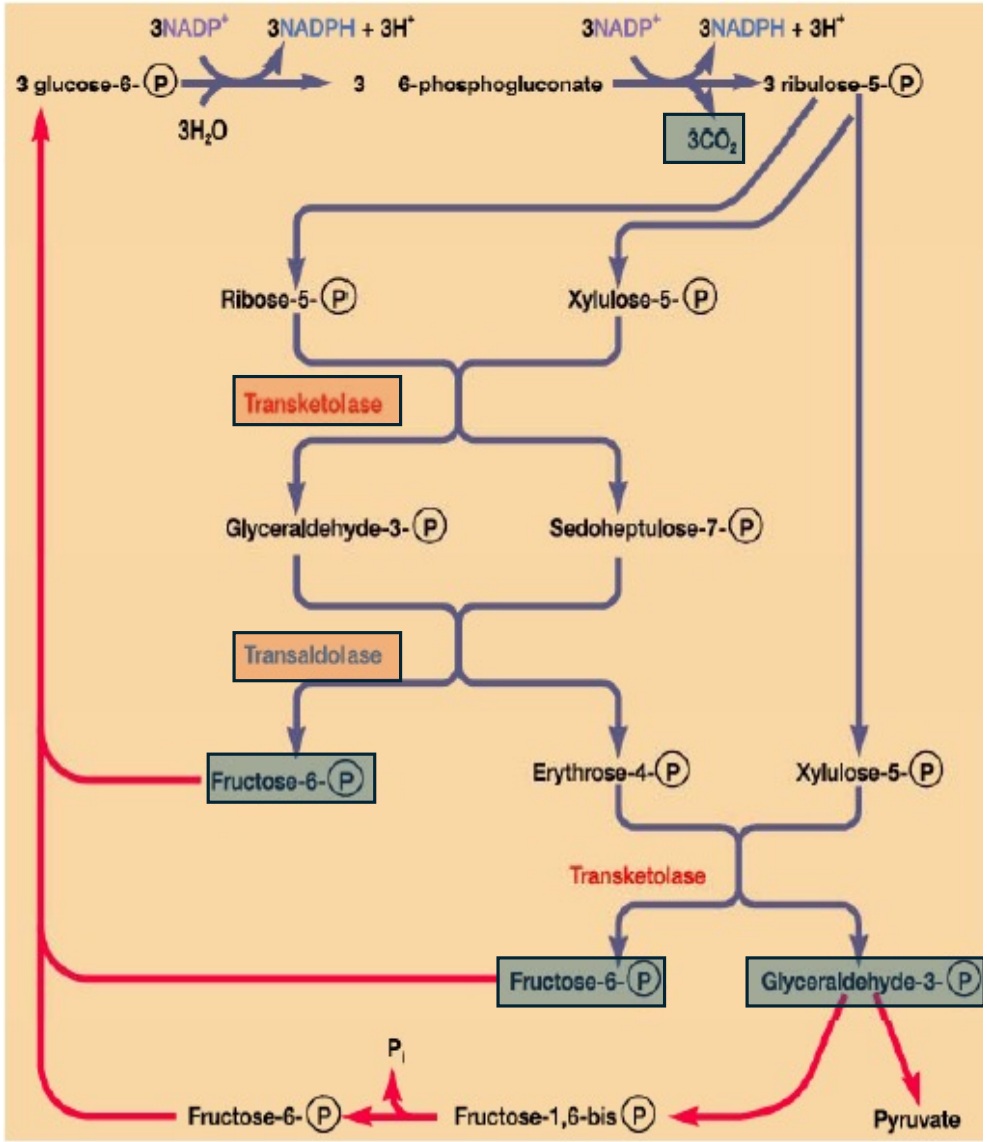
The overall result is that three G-6-P are converted to

2 fructose - 6-P

1 gly 3-P

3 $CO_2$ molecules.

6 NADPH



This pathway has several catabolic and anabolic functions which are as follows:

(1) It generates NADPH as the source of electrons required for biosynthesis.

(2) The pathway also synthesizes four and five carbon sugars for biosynthesis. It forms sedoheptulose -7-P and Erythrose-4 –P which are biosynthetic precursors to the aromatic amino acids. Ribose-5-P is a major component of nucleic acids.

(3) These intermediates may also be used to produce ATP. eg. Glyceraldehyde-3-phosphate from the pathway can enter glycolysis resulting in the formation of pyruvate and ATP. The pyruvate may further be oxidized in TCA cycle to provide more energy.

# The Entner- Doudoroff pathway

Besides the EMP pathway, another important pathway was found to be used by a large number of bacteria for carbohydrate breakdown. It was first discovered by **Entner and Doudoroff** in *Pseudomonas saccharophila*.

Now, this pathway is known to be widespread among Gram-negative bacteria e.g. *Pseudomonas, Rhizobium, Azotobacter, Agrobacterium etc.*

The **key enzymes** of ED pathway are:

1) 6- phosphogluconate dehydratase
2) 2-keto – 3- deoxy – 6 – phosphogluconate (KDPG) aldolase.

Some microorganisms e.g. *E. coli* when grown on substrates like gluconate, mannonate, or hexuronates use ED pathway for their degradation.

# Entner-Doudoroff pathway

This pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.

- Uses 6-phosphogluconate dehydratase and 2-keto-3-deoxyphosphogluconate aldolase to create pyruvate from glucose.
- Most of gram –ve bacteria like pseudomonas, rhizobium, agrobacterium.
- Produces 1 molecule NADH, 1 molecule NADPH and 1 molecule of ATP

*Stuart Hogg Essential microbiology 2nd edition John Wiley & Sons.Ltd 2005*

In this pathway, glucose-6- phosphate is first dehydrogenated to yield 6-phosphogluconate.

This is converted by a dehydratase and an aldolase reaction into one molecule of glyceraldehyde 3- phosphate and 1 molecule of pyruvate.

The glyceraldehyde 3-phosphate can then be oxidized to pyruvate by the enzymes of the EMP pathway.

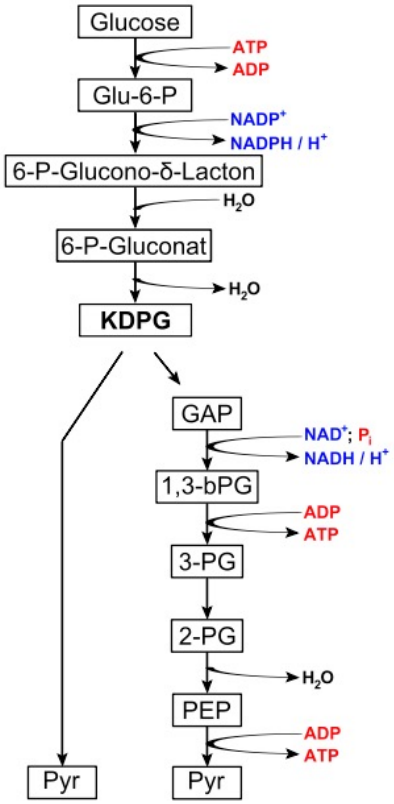



Figure: ***The Entner–Doudoroff Pathway***: *This is a diagram of the Entner-Doudoroff pathway (KDPG: 2-keto-3-deoxy-6-phosphogluconate).*

## Pentose phosphate pathway

3 Glucose-6-P

→

2 fructose-6-P + 1 gly 3-P + 3 $CO_2$ molecules + 6 NADPH

## The Entner–Doudoroff pathway

Glucose

→

2-keto-3-deoxyphosphogluconate aldolase

→

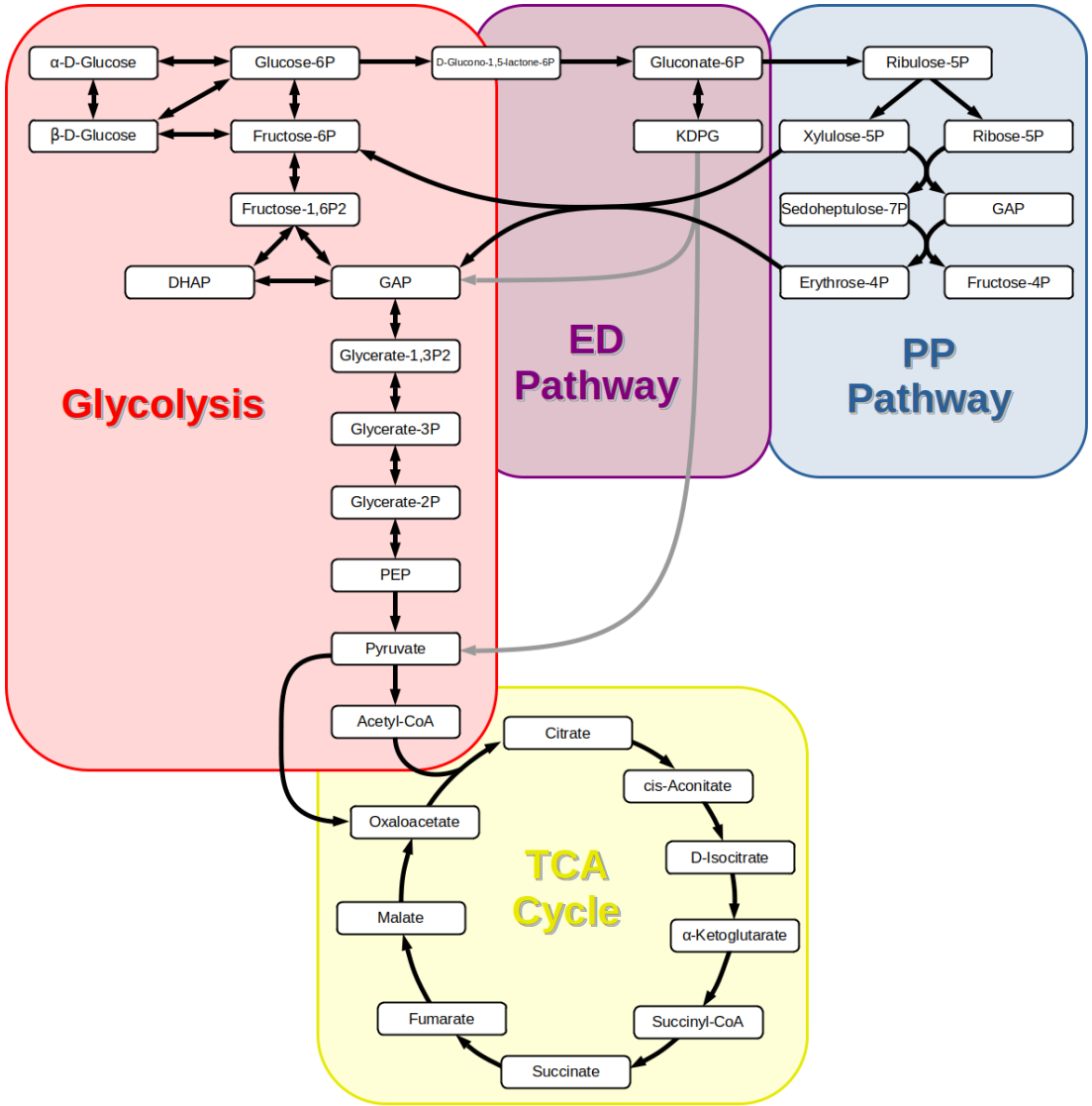
Pyruvate + gly 3-P + 1 ATP + 1 NADH + 1 NADPH.

## Glycolysis

Glucose

→

2Pyruvate + 2 ATP + 2 NADH



# Substrate level phosphorylation

In substrate level phosphorylation, ATP is formed from ADP by transfer of a high energy phosphate group from an intermediate of a fueling pathway.

For example:

2-phosphoglyceric acid → phosphoenol pyruvate → pyruvic acid + ATP

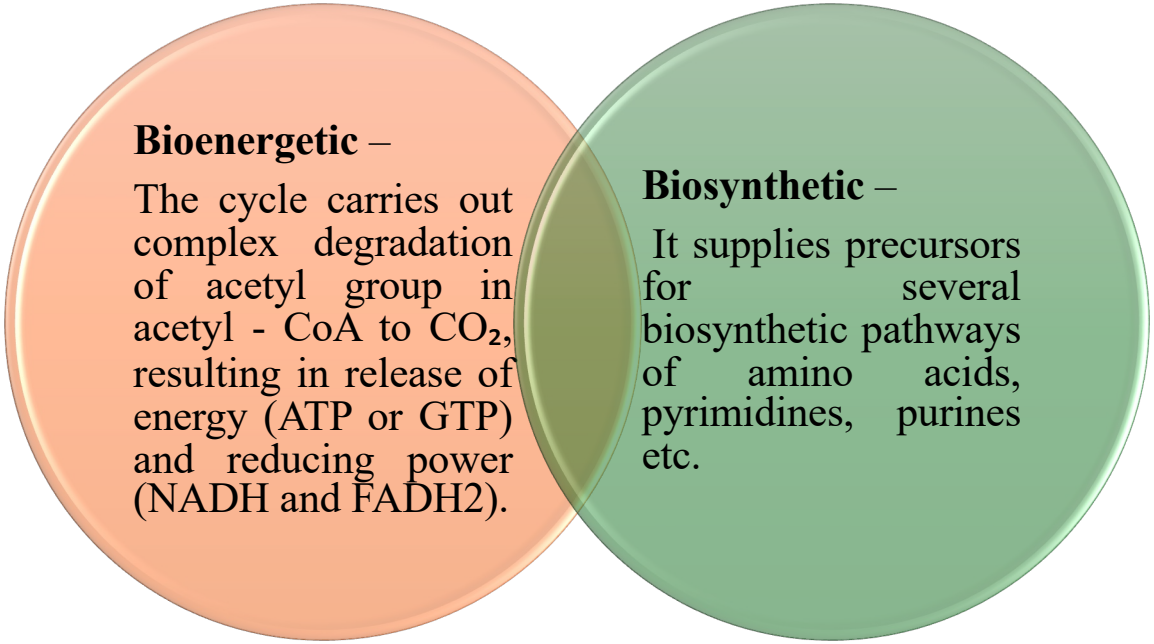
As a cosequence of the removal of a molecule of water, the low energy ester linkage of phosphate in 2-phosphoglyceric acid is converted to the high energy enol linkage in phosphoenol pyruvic acid. The high energy linked phosphate can than be transferred to ADP, the consequence of which is the generation of a molecule of ATP.

# Aerobic respiration

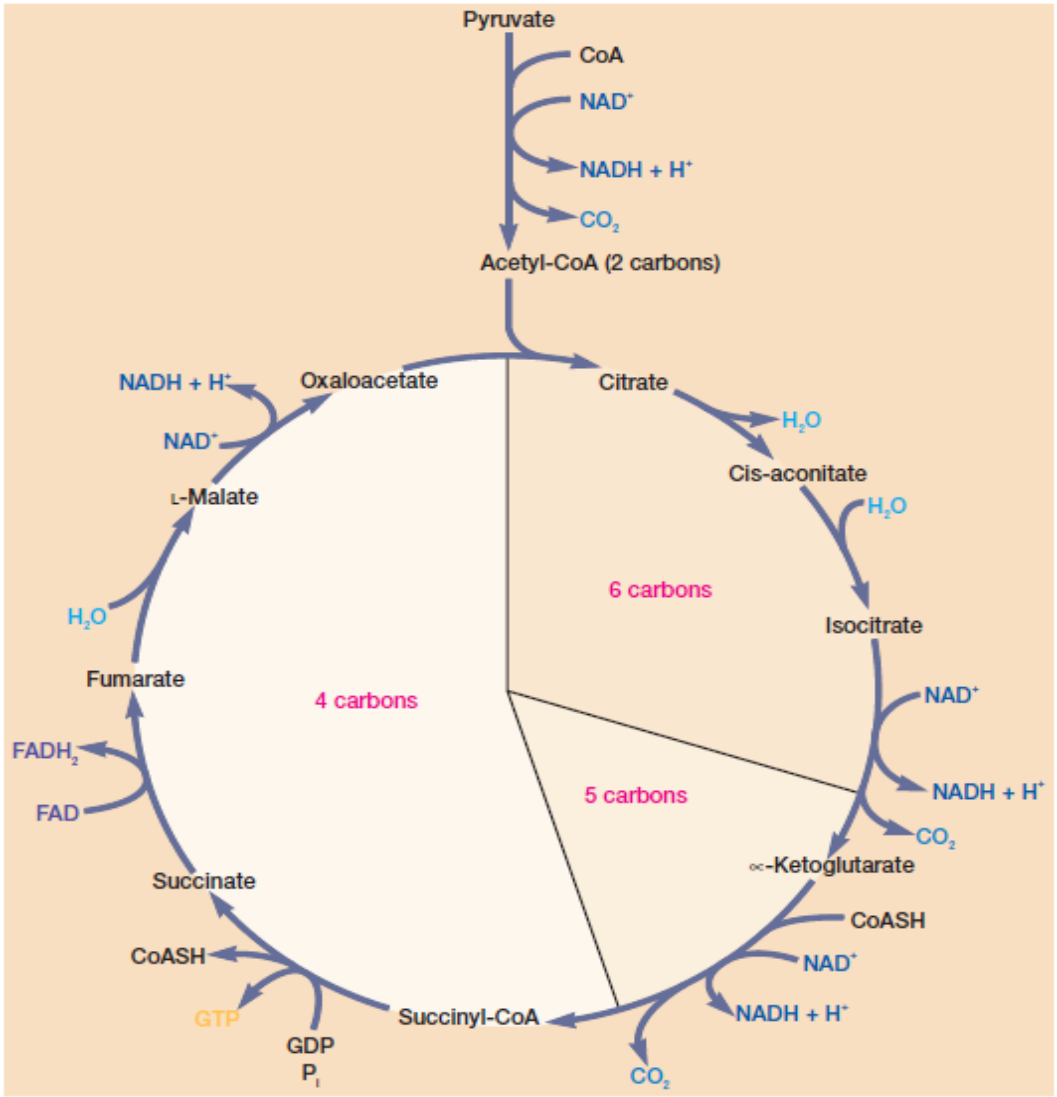
- Oxygen serves as the terminal electron acceptor for the electron-transport chain in aerobic respiration
- The aerobic respiration in bacteria typically occurs in three principal stages
  - Glycolysis
  - Krebs cycle
  - Electron transport chain

# The Tricarboxylic Acid (TCA) Cycle (Phase III)

The TCA cycle was first discovered by **Eggleston** and **Krebs** in animal tissues. It is also called as **Krebs** or **Citric acid** cycle. The cycle is considered as central pathway of aerobic metabolism as it serves 2 purposes:

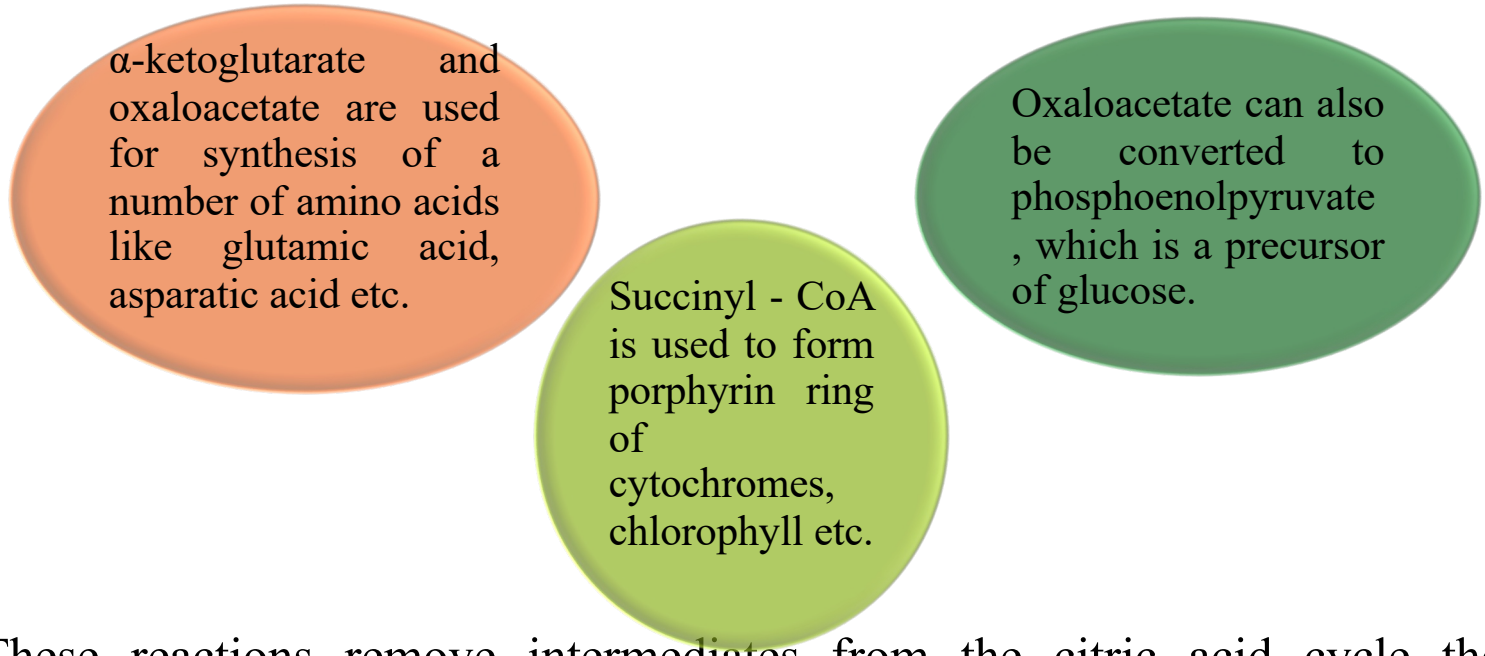



$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + Pi + 2H_2O \longrightarrow 2CO_2 + 3NADH + 3H^+ + FADH_2 + GTP + CoA$$




**The Tricarboxylic Acid Cycle.** The cycle may be divided into **three stages** based on the size of its intermediates. The three stages are separated from one another by two decarboxylation reactions (reactions in which carboxyl groups are lost as $CO_2$). The pyruvate dehydrogenase complex forms acetyl-CoA through pyruvate oxidation.

TCA cycle is also an important source of biosynthetic precursors e.g.



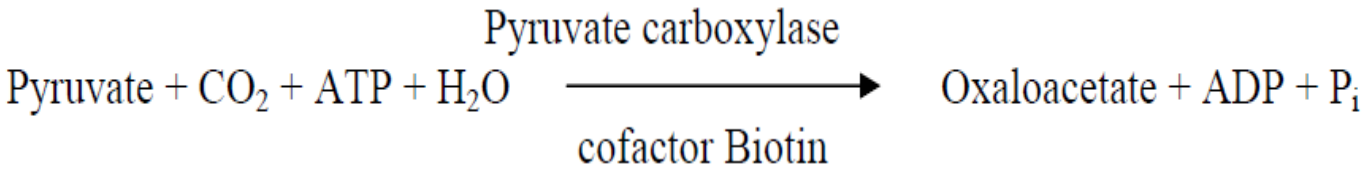

These reactions remove intermediates from the citric acid cycle thereby effecting its efficiency. It has been found that microorganisms have some reactions that re-supply these intermediates to the TCA cycle.

Such reactions that replace cycle intermediates are called as **anapleurotic ("to fill in") reactions**.

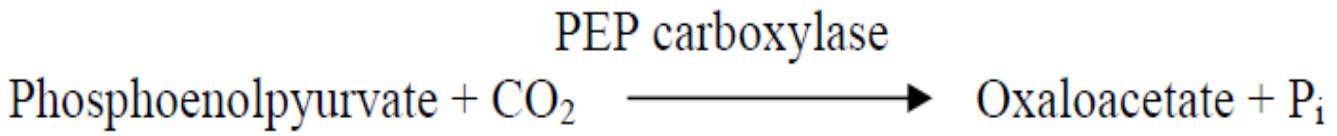
Usually these reactions involve $CO^2$ fixation.

Various microorganisms use different enzymes for this purpose, e.g.

(i) *Arthrobacter globiformis* and yeasts:




(ii) *E. coli* and *Salmonella typhimurium:*



# Glyoxylate cycle

Glyoxylate cycle is a variation of the TCA cycle.

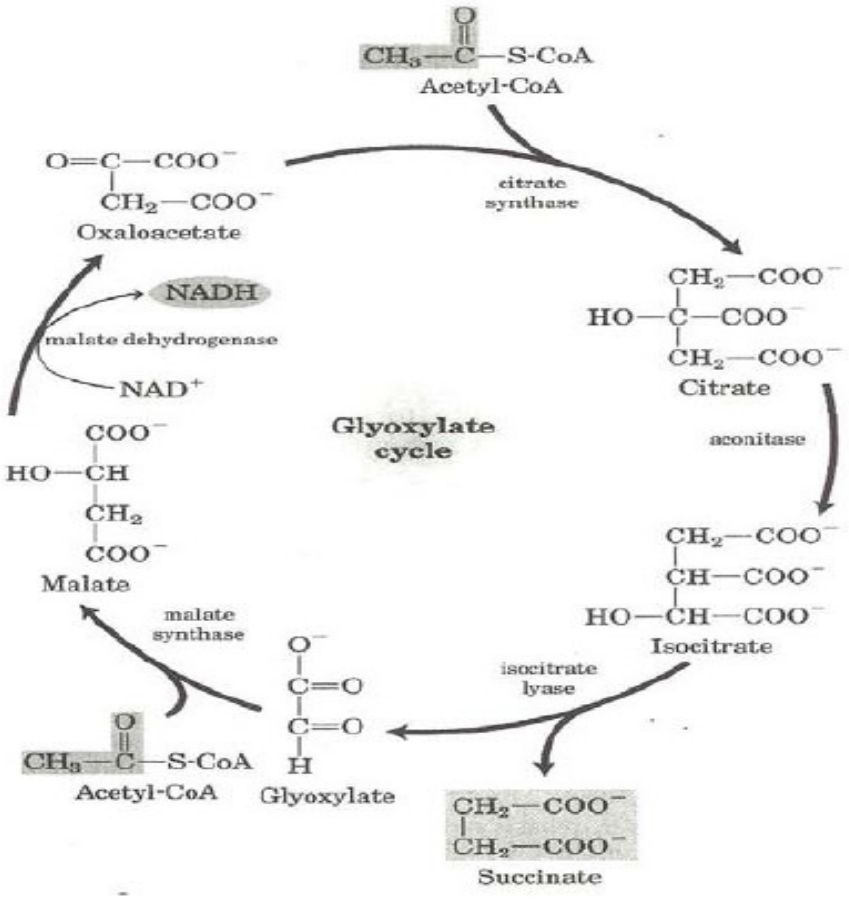
It is so called because glyoxylate is an important **intermediate** of this cycle. It is present in those organisms, who have the ability to **grow** on **acetate** (a $C^2$ compd) and **long chain fatty acids** (which generate $C^2$ compds on metabolism) etc.

In these organisms also, the anabolic reactions remove intermediates from the TCA cycle. But they do not have three carbon compounds like phosphoenolpyruvate, which can be converted to oxaloacetate by anapleurotic reactions.

In such cases, the oxaloacetate needed to continue the cycle is produced through the **glyoxylate** cycle.

The cycle consists of most of the enzymes of TCA cycle. It has two additional enzymes: (i) isocitrate lyase which splits isocitrate to succinate and glyoxylate and ii) malate synthase combines glyoxylate and acetyl-CoA to malate.

When succinate or other intermediates are removed from the cycle for biosynthesis, the malate synthesized from glyoxylate and acetyl-CoA gets converted to oxaloacetate, thereby continuing the cycle.



# Electron Transport Chain

During complete oxidation of one molecule of glucose, only four ATP molecules are synthesized. In addition, there is formation of NADH and $FADH^2$, the reducing powers. It is the oxidation of these reducing powers via respiratory / electron transport chain (ETC) which generates most ATPs.

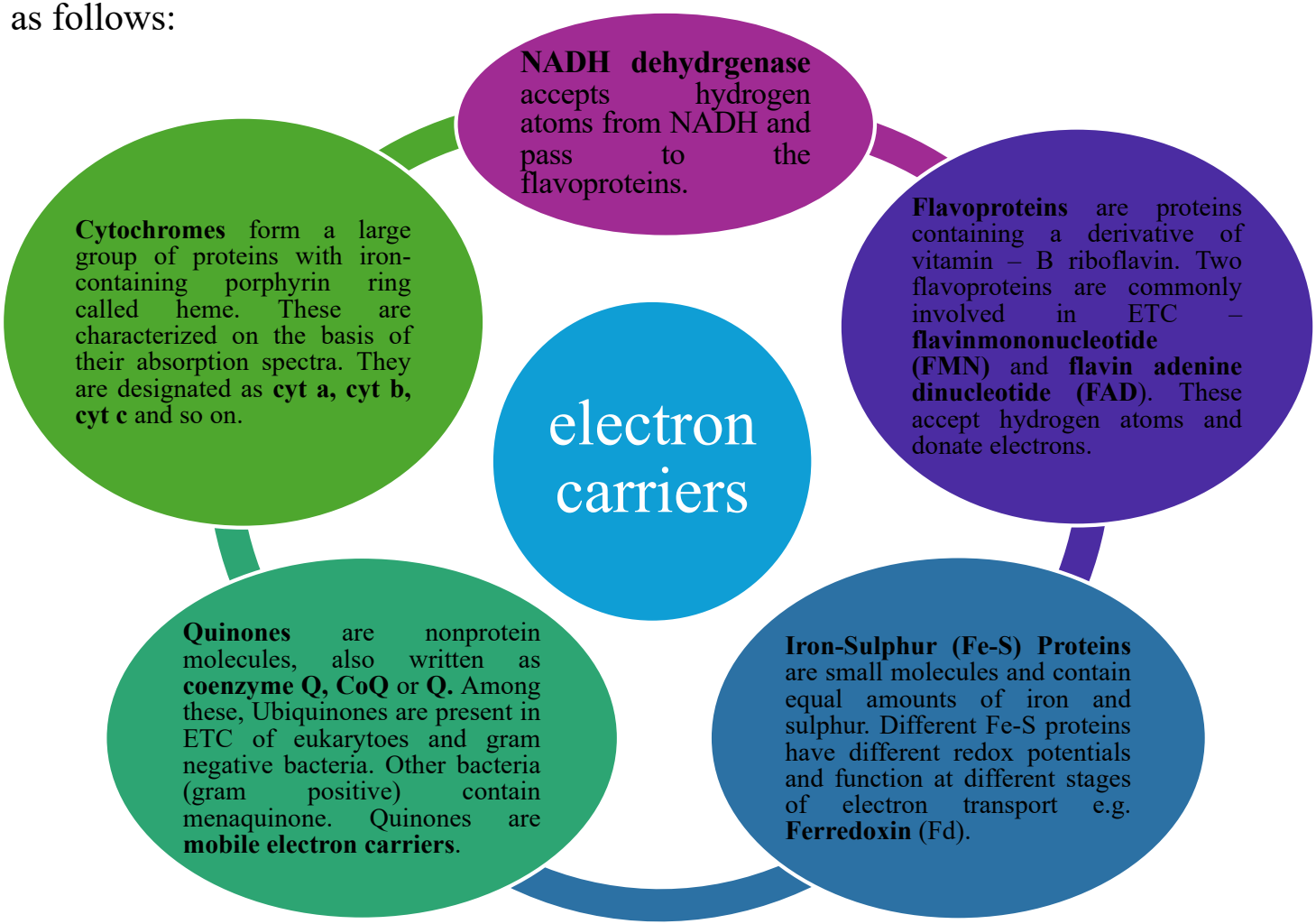
The ETC is composed of a series of biomolecules which perform following functions:

(i) They act as electron carriers. That is they accept electrons from an electron donor and transfer them to an electron acceptor.

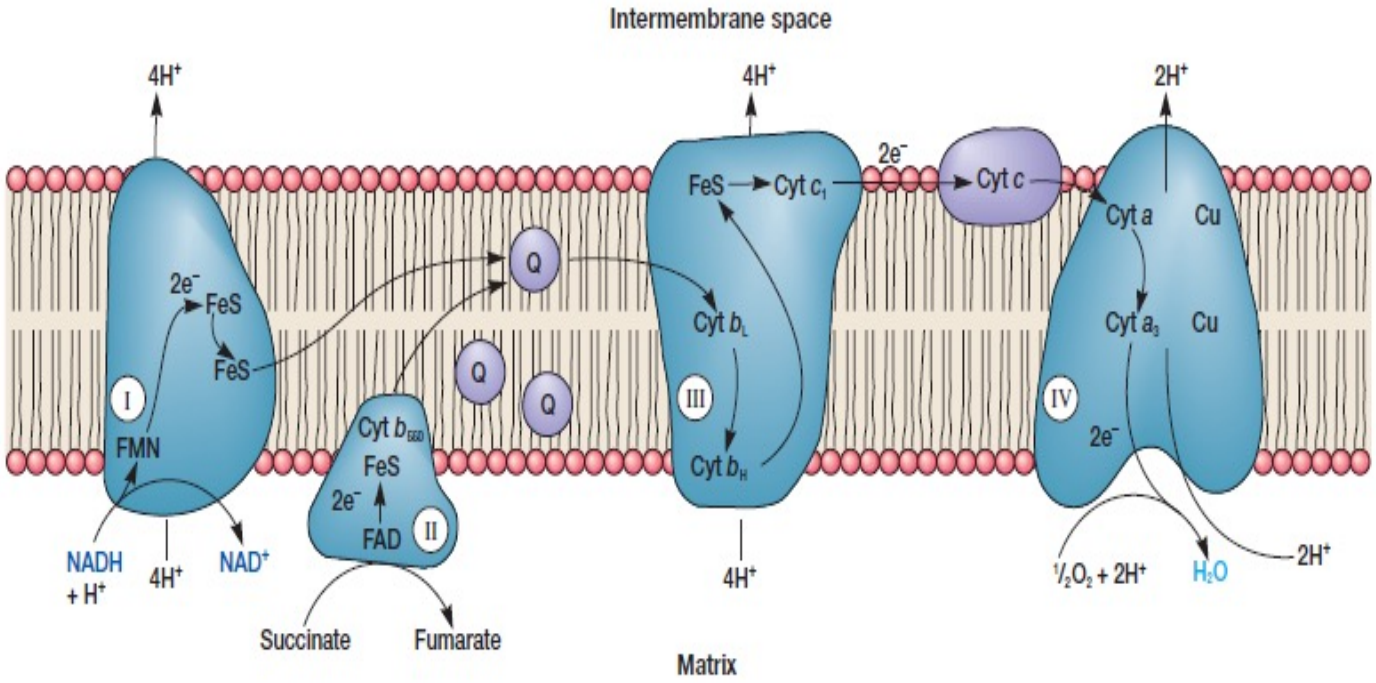
(ii) They conserve some of the energy released during electron transfer for synthesis of ATP.

Various electron carriers in ETC include NADH dehydrogenase, Flavoproteins, Cytochromes, Quinones, Iron-sulphur proteins etc.

The electron carriers in the ETC belong to different classes of biomolecules and are as follows:



In mitochondria, the components are present within its inner membrane. They are arranged in four complexes: NADH – CoQ reductase (Complex I), Succinate – CoQ (Complex II), cytochrome *c* reductase (Complex III) and cytochrome *c* oxidase (Complex IV).




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.

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.

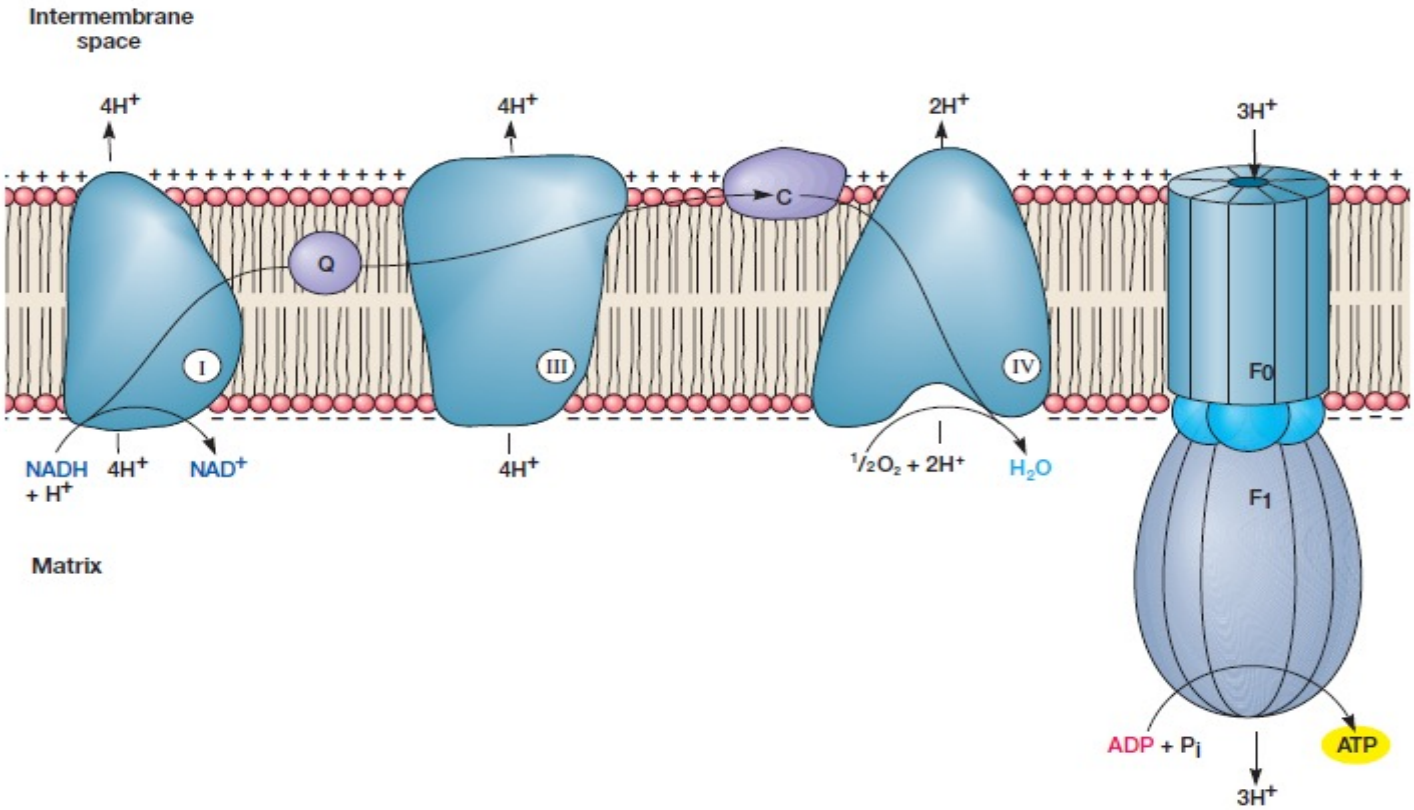
Coenzyme Q transports electrons from complexes I and II to complex III.

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.

# Oxidative phosphorylation

Energy is released during the electron transfers from NADH (or $FADH^2$) to molecular oxygen. This energy is used to synthesize ATP by adding an inorganic phosphate to ADP (phosphorylation). This process is called as **oxidative phosphorlyation**.




The flow of electrons from NADH to oxygen causes protons to move from the **mitochondrial matrix to the intermembrane space**. This generates **proton** and **electrical gradients**. When protons move back to the matrix through the **F1F0** complex, F1 synthesizes **ATP**. In prokaryotes, the process is similar except that the protons move from the **cytoplasm to the periplasm**.

ATP Yield from the Aerobic Oxidation of Glucose by Eukaryotic Cells

| | |
|---|---|
| **Glycolytic Pathway** | |
| Substrate-level phosphorylation (ATP) | 2 ATP[a] |
| Oxidative phosphorylation with 2 NADH | 6 ATP |
| **2 Pyruvate to 2 Acetyl-CoA** | |
| Oxidative phosphorylation with 2 NADH | 6 ATP |
| **Tricarboxylic Acid Cycle** | |
| Substrate-level phosphorylation (GTP) | 2 ATP |
| Oxidative phosphorylation with 6 NADH | 18 ATP |
| Oxidative phosphorylation with 2 $FADH_2$ | 4 ATP |
| **Total Aerobic Yield** | 38 ATP |

# Anaerobic Respiration

Many bacteria have electron transport chains that can operate with exogenous electron acceptors other than $O_2$. This energy-yielding process is called anaerobic respiration. The major electron acceptors are nitrate, sulfate, and $CO_2$, but metals and a few organic molecules can also be reduced

Some bacteria can use nitrate as the electron acceptor at the end of their electron transport chain and still produce ATP. Often this process is called **dissimilatory nitrate reduction.** Nitrate may be reduced to nitrite by nitrate reductase, which replaces cytochrome oxidase.

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

The nitrite formed is also quite toxic. Therefore nitrate often is further reduced all the way to nitrogen gas, a process known as **denitrification.** Each nitrate will then accept five electrons, and the product will be nontoxic.

$$2NO_3^- + 10e^- + 12H^+ \longrightarrow N_2 + 6H_2O$$

Denitrification is a multistep process with four enzymes participating: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase.

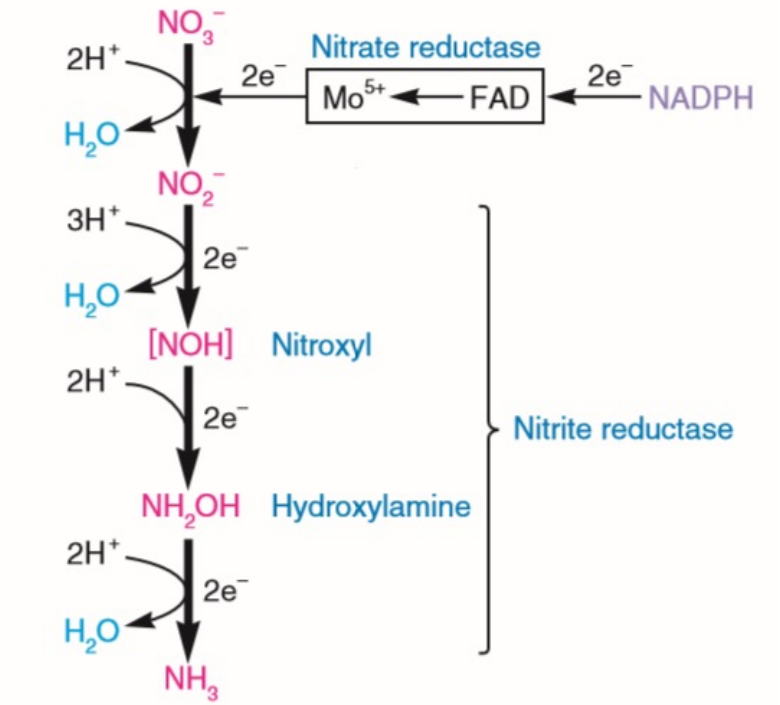
$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

# NITRATE REDUCTION

- The reduction of nitrate into ammonia and its incorporation in organic material is known as **assimilatory nitrate reduction**.
- The nitrogen in nitrate ( $NO_3^-$ ) is much more oxidized than that in ammonia. Therefore, nitrate must first be reduced to ammonia before the nitrogen can be converted into an organic form.
- This process is widespread among bacteria, fungi, and photosynthetic protists and it is an important step in nitrogen cycle.

**NITRATE REDUCTION CONTINUED.....**

- Assimilatory nitrate reduction takes place in **cytoplasm** in bacteria.




- The ammonia is then incorporated into amino acids.

# SULFATE REDUCTION

- Sulfur is needed for the synthesis of the amino acids cysteine and methionine.
- The sulfur atom in sulfate is more oxidized than it is in cysteine and other organic molecules; thus sulfate must be reduced before it can be assimilated. This process is known as **assimilatory sulfate reduction.**

- Assimilatory sulfate reduction involves sulfate activation through the formation of **phosphoadenosine 5'-phosphosulfate**, followed by reduction of th

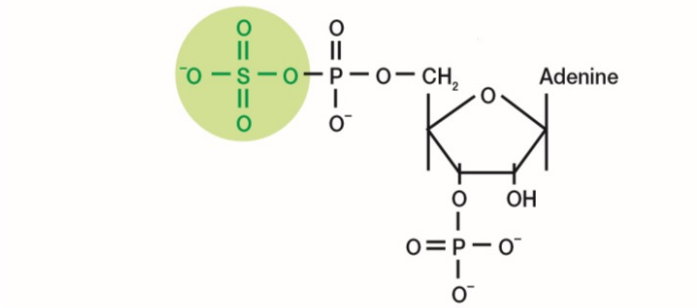
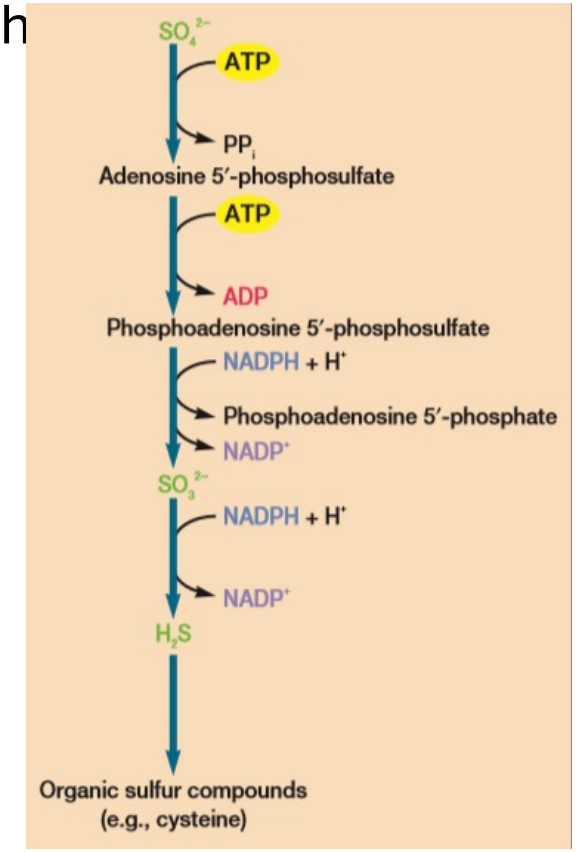
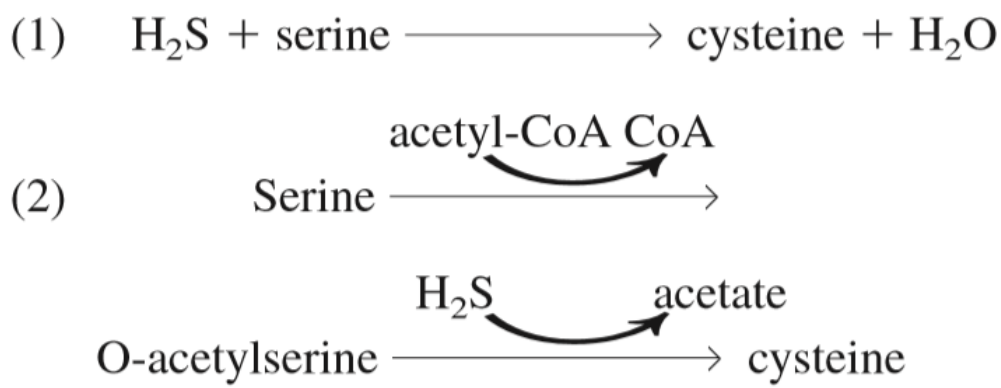



**Figure 10.8** **Phosphoadenosine 5′-phosphosulfate (PAPS).** sulfate group is in color.



- Cysteine can be synthesized from hydrogen sulfide in two ways. Fungi appear to combine hydrogen sulfide with serine to form cysteine (process 1), whereas many bacteria join hydrogen sulfide with O- acetylserine instead (process 2).




- Once formed, cysteine can be used in the synthesis of other sulfur- containing organic compounds.

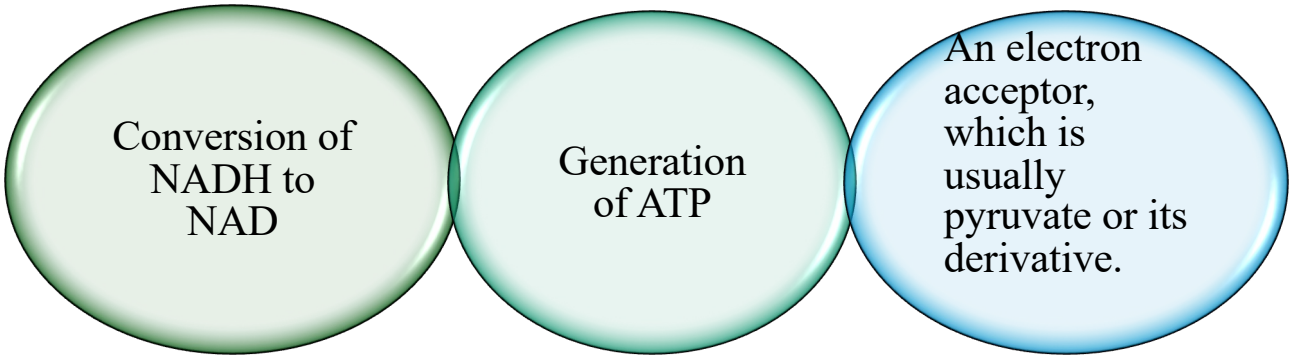
# Fermentation

In the absence of aerobic or anerobic respiration, NADH is not oxidized by the ETC. This is because no external electron acceptor is available.

But to continue the metabolism, NAD must be regenerated. In such situations, microorganisms do not convert pyruvate into Acetyl - CoA. Instead, they use pyruvate or its derivatives as an electron acceptor for reoxidation of NADH. It also leads to production of ATP.

This process of extraction of energy from organic substrates in the absence of oxygen is called **fermentation**.

the process of fermentation includes:



- In biochmistry, fermentation is an enzyme-catalyzed energy-generating process in which organic compounds act as both donors and acceptors of electrons.

- In microbiology, Fermentation which involves the conversion of substrates to desired product with the help of microorganisms

There are many types of fermentations.

They are usually classified on the basis of main **end products** e.g.

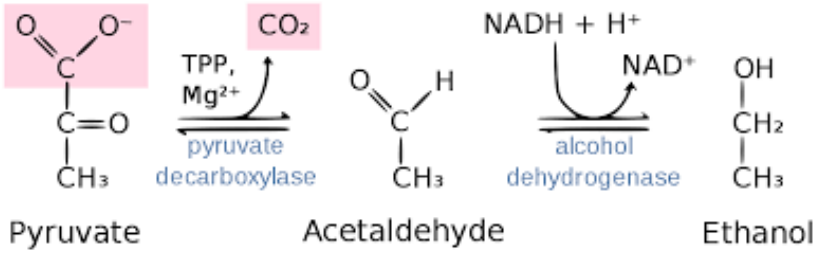
**alcohol fermentation**,
**lactic acid fermentation**,
**methane fermentation**,
**butyric acid fermentation**.

# Anaerobic respiration in Fungi e.g. Yeast

Pyruvate → Acetaldehyde + $CO_2$ enzyme: pyruvate decarboxylase

Acetaldehyde + NADH + $H^+$ → Ethanol + $NAD^+$ enzyme: alcohol dehydrogenase

Overall: Pyruvate → Ethanol + $CO_2$




Ethanol is the alcohol found in alcohol drinks.

This process is known as alcohol fermentation and its occurrence in yeast is made use of in the manufacture of beer, wine and other alcohol drinks.

Production of $CO_2$ by yeast is used in bread making, to make dough rise.

Ethanol is a waste product that still contains a lot of energy (e.g. it is used to make Gasohol, a fuel that is used for cars in Brazil).

Overall two molecules of ATP are produced per glucose molecule

# Alcohol fermentation

Most of the alcohol fermentation in nature and in industry is carried out by yeast belonging to *Saccharomyces* species. These organisms ferment sugars to ethanol and $CO_2$ by the following equation:

$$\underset{\text{(Sugar)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(Ethanol)}}{2\,C_2H_5OH} + 2\,CO_2$$

Yeasts employ EMP pathway for glucose breakdown. The pyruvate produced is decarboxylated to acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase. The NADH produced during glycolysis acts as the electron donor.

Only two ATPs are produced per molecule of glucose during this fermentation. Some strictly anaerobic bacterial species also carry out alcohol fermentation e.g. *Zymomonas mobilis, Sarcina ventriculi, Erwinia amylovora.* However, they also form small quantities of other products like acetate, molecular hydrogen, lactate, etc.

# Lactic acid fermentation

Lactic acid is a very common end product of bacterial fermentations. The genera which produce large amounts of lactate are called lactic acid bacteria.

All lactic acid bacteria are aerotolerant anaerobes i.e. they are facultative aerobes that grow in the presence of oxygen but do not use it in respiration. Instead, they produce energy by fermentation of sugars.

# Anaerobic respiration in animals, muscle tissue

Pyruvate + NADH + $H^+$ → Lactate + $NAD^+$ enzyme: lactate dehydrogenase

Note that no $CO_2$ is produced unlike in fungi. Also, alcohol is not made.

Instead, the product is lactate whose build up in muscles contributes to the sensation of fatigue and can contribute to cramp.

As with anaerobic respiration in fungi, overall only two molecules of ATP are produced per glucose molecule and the waste product, lactate, still contains a lot of energy.

Overall: Glucose → (animal or musle tissue) 2 lactate + 2 ATP

Glucose → (Fungi) 2ethanol + $2CO_2$ + 2 ATP

# METHANOGENESIS

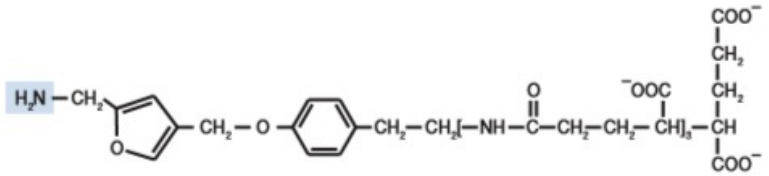
- Methanogens are strict anaerobes that obtain energy by converting $CO_2$, $H_2$, formate, methanol, acetate, and other compounds to either methane or methane and $CO_2$. This process is called **methanogenesis.**
- This is the largest group of archaea. There are five orders (Methanobacteriales, Methanococcales, Metha- nomicrobiales, Methanosarcinales, and Methanopyrales) and 26 genera.

**Table 20.2** Selected Characteristics of Representative Genera of Methanogens

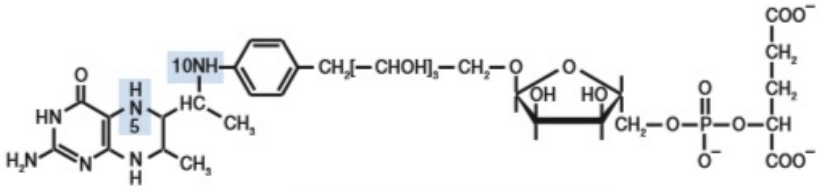
| Genus | Morphology | % G + C | Wall Composition | Gram Reaction | Motility | Methanogenic Substrates Used |
|---|---|---|---|---|---|---|
| Order *Methanobacteriales* | | | | | | |
| *Methanobacterium* | Long rods or filaments | 32–61 | Pseudomurein | + to variable | – | $H_2 + CO_2$, formate |
| *Methanothermus* | Straight to slightly curved rods | 33 | Pseudomurein with an outer protein S-layer | + | + | $H_2 + CO_2$ |
| Order *Methanococcales* | | | | | | |
| *Methanococcus* | Irregular cocci | 29–34 | Protein | – | – | $H_2 + CO_2$, formate |
| Order *Methanomicrobiales* | | | | | | |
| *Methanomicrobium* | Short curved rods | 45–49 | Protein | – | + | $H_2 + CO_2$, formate |
| *Methanogenium* | Irregular cocci | 52–61 | Protein or glycoprotein | – | – | $H_2 + CO_2$, formate |
| *Methanospirillum* | Curved rods or spirilla | 45–50 | Protein | – | + | $H_2 + CO_2$, formate |
| *Methanosarcina* | Irregular cocci, packets | 36–43 | Heteropolysaccharide or protein | + to variable | – | $H_2 + CO_2$, methanol, methylamines, acetate |

**METHANOGENESIS CONTINUED……**

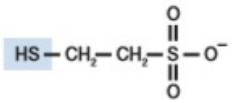
- As might be inferred from the methanogens' ability to produce methane anaerobically, their metabolism is unusual. These prokaryotes contain several unique cofactors: **tetrahydromethanopterin ($H_4$MPT), methanofuran (MFR), coenzyme M(2-mercaptoethanesulfonic acid), coenzyme $F_{420}$, and coenzyme $F_{430}$.**



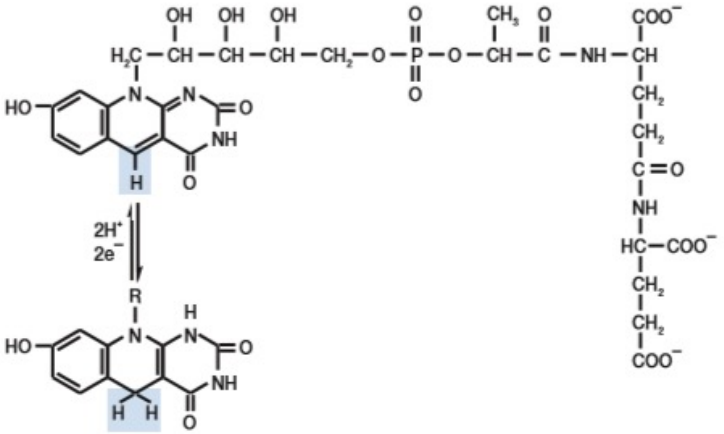
(a) Methanofuran (MFR)



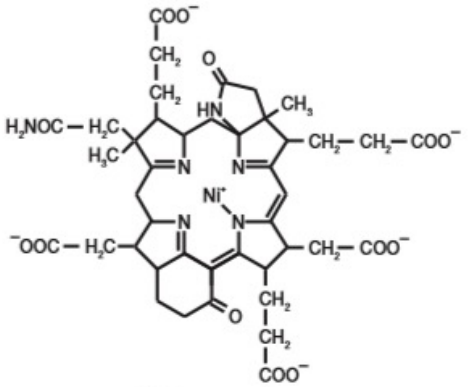
(b) Tetrahydromethanopterin ($H_4$MPT)



(c) Coenzyme M



(d) Coenzyme $F_{420}$



(e) Coenzyme $F_{430}$

**Figure 20.11 Methanogen Coenzymes.** The portion of $F_{420}$ (**d**) that is reversibly oxidized and reduced is shown in color. MFR (**a**), $H_4$MPT (**b**), and coenzyme M (**c**) carry one-carbon units during methanogenesis (MFR and MPT also participate in the synthesis of acetyl-CoA). The places where the carbon units are attached are in color. $H_4$MPT carries carbon units on nitrogens 5 and 10 in the same way as the coenzyme tetrahydrofolate. Coenzyme $F_{430}$ (**e**) is a coenzyme for methyl-CoM methylreductase.

**METHANOGENESIS CONTINUED.....**

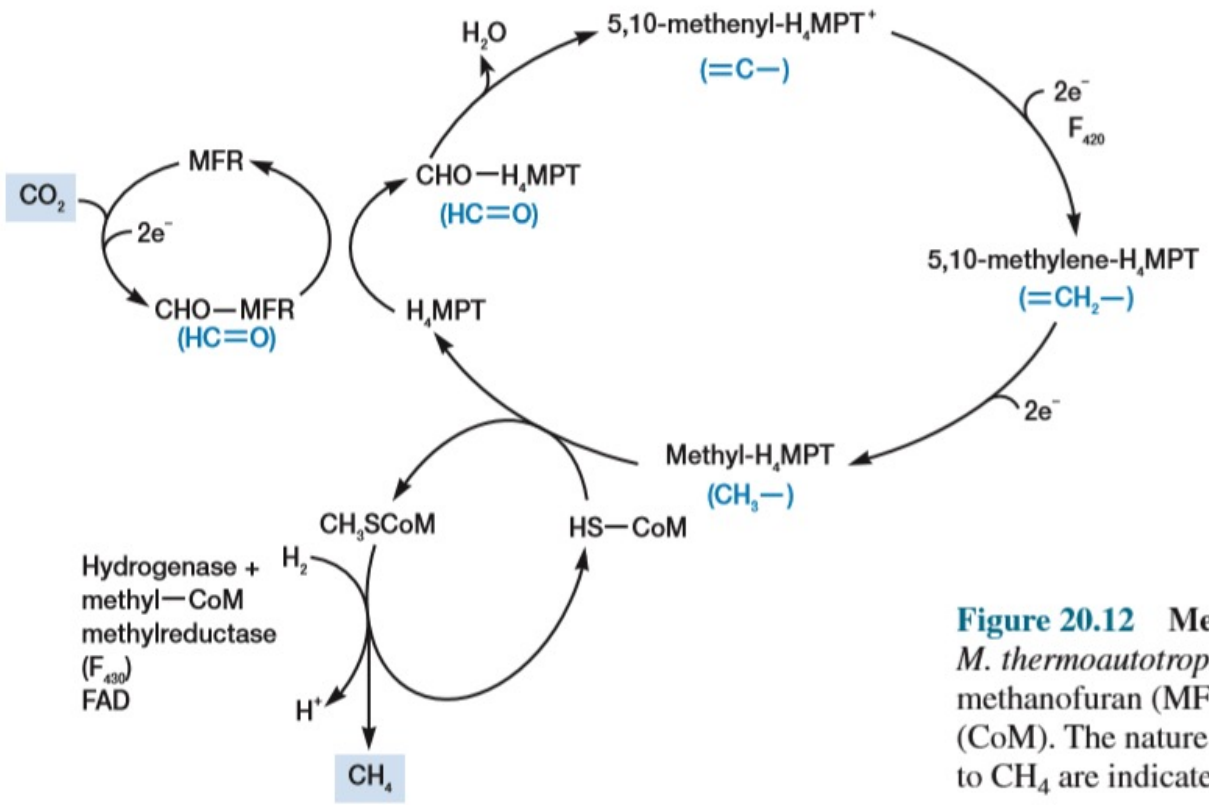



**Figure 20.12** **Methane Synthesis.** Pathway for $CH_4$ synthesis from $CO_2$ in *M. thermoautotrophicum.* Cofactor abbreviations: methanopterin (MPT), methanofuran (MFR), and 2-mercaptoethanesulfonic acid or coenzyme M (CoM). The nature of the carbon-containing intermediates leading from $CO_2$ to $CH_4$ are indicated in parentheses. See text for further details.

- It appears that ATP synthesis is linked with methanogenesis by electron transport, proton pumping, and a chemiosmotic mechanism.
- In addition, the transfer of methyl groups from methyl-$H_4$MPT to HS-CoM releases sufficient energy for the uptake of the sodium ions. This results in a sodium motive force that could also drive ATP synthesis.

# ACETOGENESIS

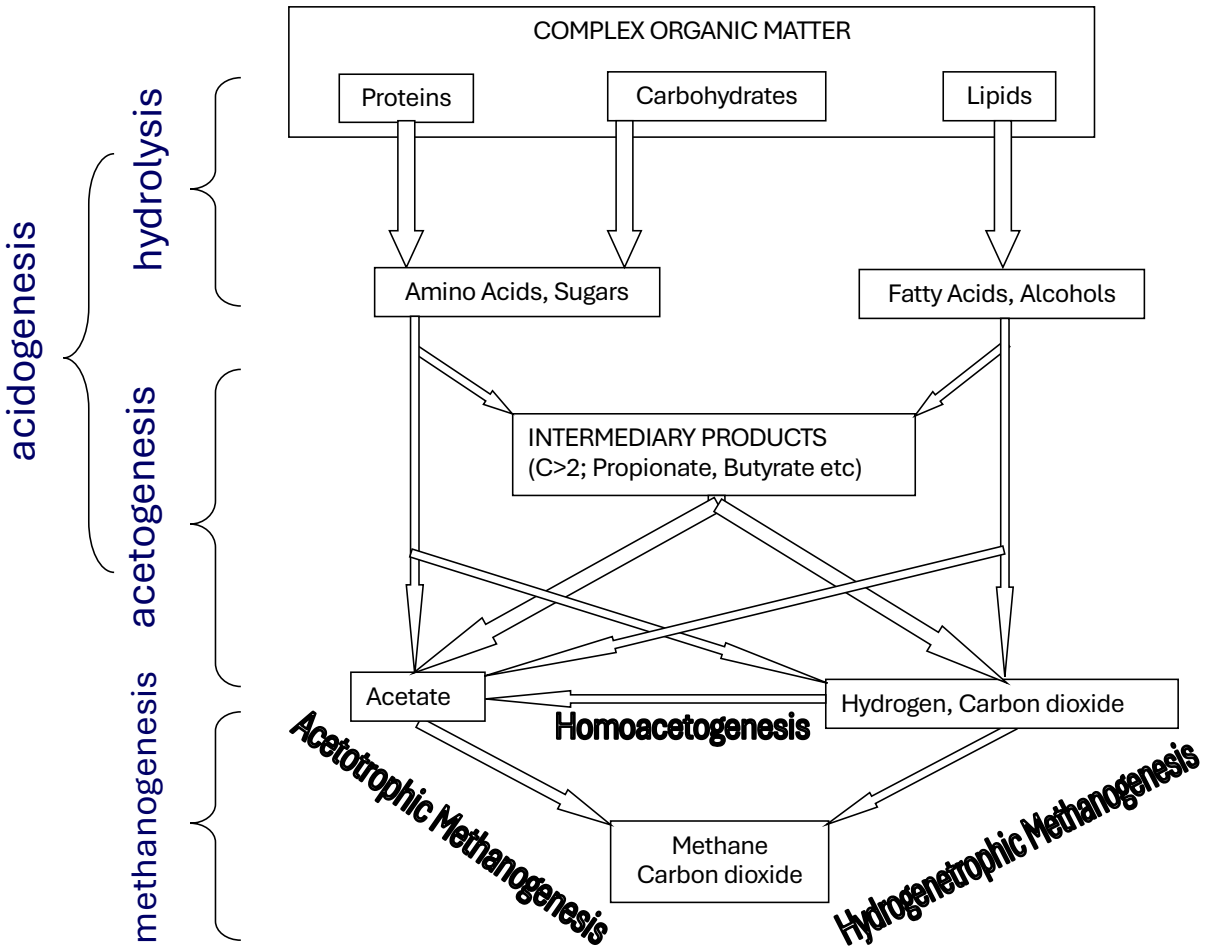
- **Acetogenesis** is a process through which acetate is produced from $CO_2$ and an electron source (e.g., $H_2$, CO, formate, etc.) by anaerobic bacteria via the reductive acetyl-CoA or Wood-Ljungdahl pathway. The different bacterial species that are capable of acetogenesis are collectively termed acetogens.

## Biochemistry

- The precursor to acetic acid is the thioester acetyl CoA. The key aspects of the acetogenic pathway are several reactions that include the reduction of carbon dioxide to carbon monoxide and the attachment of the carbon monoxide to a methyl group. The first process is catalyzed by enzymes called carbon monoxide dehydrogenase. The coupling of the methyl group (provided by methylcobalamin) and the CO is catalyzed by acetyl CoA synthetase.

$$\mathbf{2\,CO_2 + 4\,H_2 \rightarrow CH_3COOH + 2H_2O}$$

## *Organics Conversion in Anaerobic Systems*



# • Microbiology

The anaerobic degradation of complex organic matter is carried out by a series of bacteria and archae .There exists a coordinated interaction among these microbes.

## ✓FERMENTATIVE BACTERIA

This group of bacteria is responsible for the first stage of anaerobic digestion - hydrolysis and acidogenesis. These bacteria are either facultative or strict anaerobes.

The anaerobic species belonging to the family of Streptococcaceae and Enterobacteriaceae and to the genera of *Bacteroides*, *Clostridium, Butyrivibrio*, *Eubacterium*, *Bifidobacterium* and *Lactobacillus* are most common.

## ✓HYDROGEN PRODUCING ACETOGENIC BACTERIA

This group of bacteria metabolizes propionate and other organic acids (>C-2), alcohols and certain aromatic compounds (i.e. benzoate) into acetate and $CO_2$.

$$CH_3CH_2COO^- \rightarrow CH_3COO^- + CO_2 + H_2$$

Syntrophic association of acetogenic organisms with methanogenic $H_2$- consuming bacteria helps to lower the concentration of $H_2$ below inhibitory level so that propionate degrading bacteria are not suppressed by excessive $H_2$ level.

## ✓HOMOACETOGENS

Homoacetogenesis has gained much attention in recent years in anaerobic processes due to its final product: acetate, which is the important precursor to methane generation.

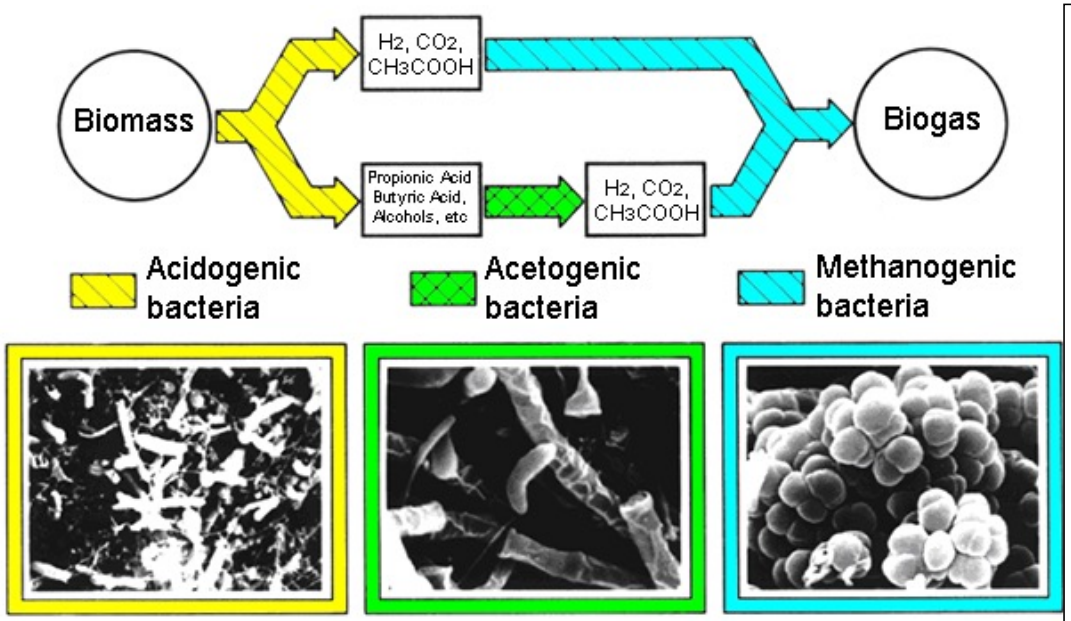
The bacteria are, $H_2$ and $CO_2$ users. *Clostridium aceticum* and *Acetobacterium woodii* are the two homoacetogenic bacteria isolated from the sewage sludge.

Homoacetogenic bacteria have a high thermodynamic efficiency; as a result there is no accumulation $H_2$ and $CO_2$ during growth on multi-carbon compounds.

$$\mathbf{CO_2 + H_2 \rightarrow CH_3COOH + 2H_2O}$$

# OBLIGATE SYNTROPHY

Both species (e.g., a methanogen and an acetogen) require the other: the acetogen provides the hydrogen; the methanogen prevents a build-up of hydrogen which inhibits the acetogens.



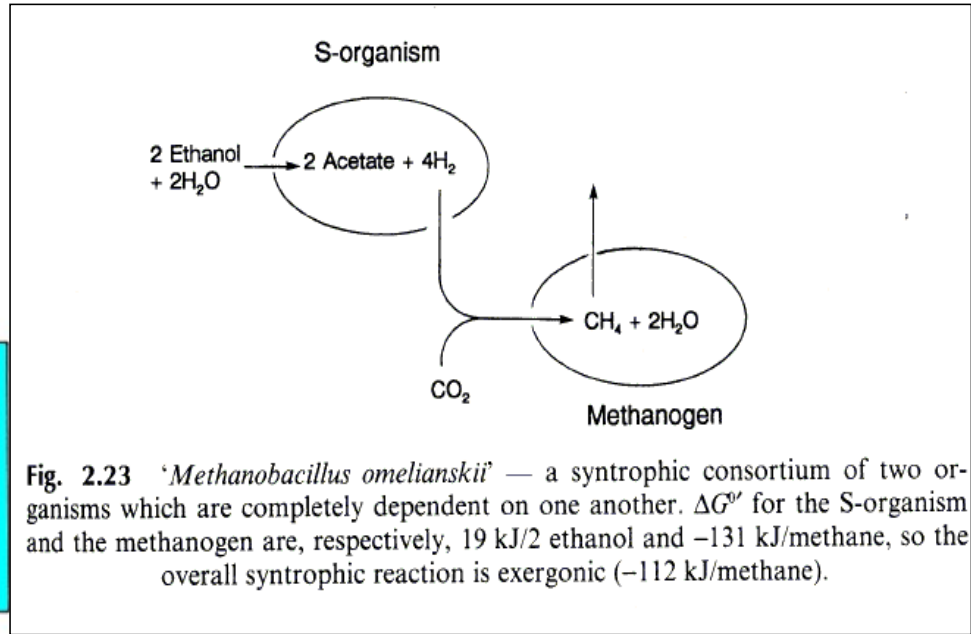




**Fig. 2.23** '*Methanobacillus omelianskii*' — a syntrophic consortium of two organisms which are completely dependent on one another. $\Delta G^{0\prime}$ for the S-organism and the methanogen are, respectively, 19 kJ/2 ethanol and −131 kJ/methane, so the overall syntrophic reaction is exergonic (−112 kJ/methane).

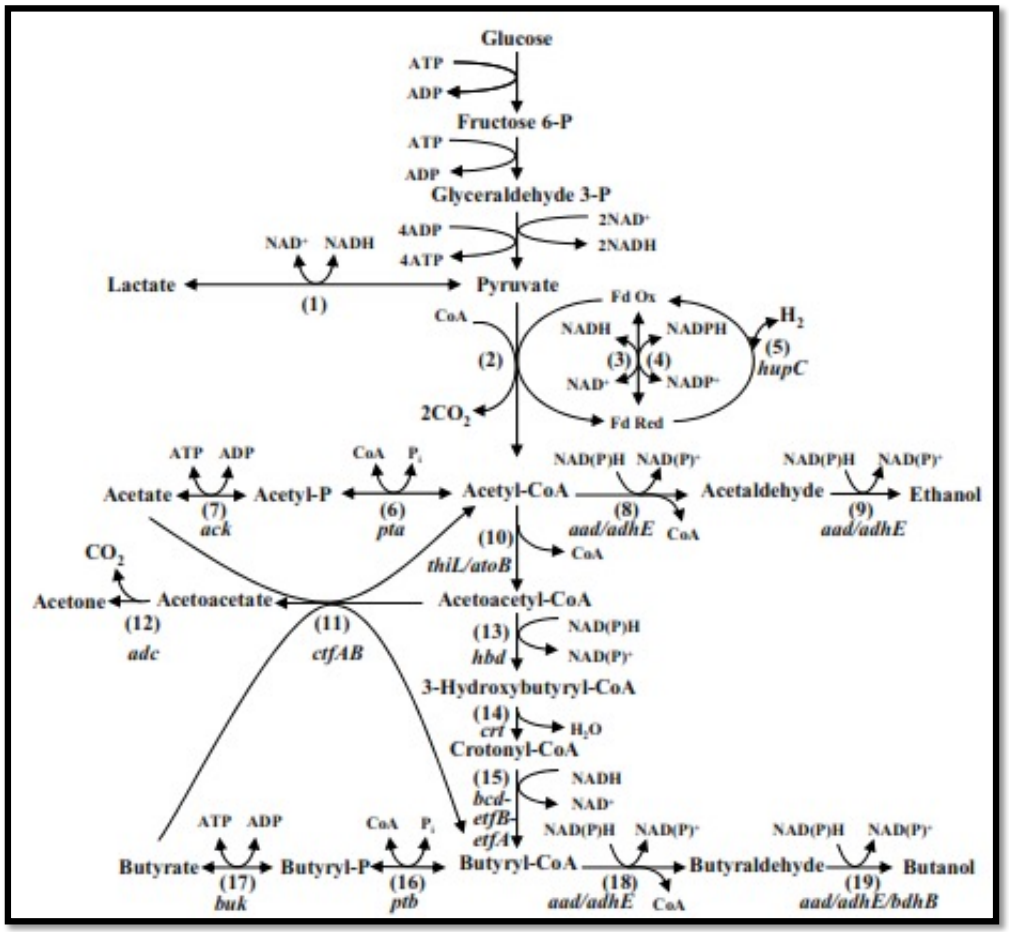
# ABE Fermentation – An Introduction

- Uses bacterial fermentation to form Acetone, Butanol and Ethanol from sugar.
- Developed by Chaim Weizmann during WW I.
- Anaerobic process.
- It produces solvents in 3:6:1 ratio where 3 parts acetone, 6 part butanol and 1 part ethanol are produced.
- Uses the strain of bacteria genus Clostridia. *Clostridium acetobutylicum* is generally used.
- Potential renewable source of energy with Butanol as biofuel.

# Metabolic stages

- It can be divided into two major and distinct phases [1]:
  - Acidogenesis
    - Occurs during exponential growth phase.
    - Formation of acetate and butyrate from acetyl – CoA and Butyryl – CoA.
  - Solventogenesis
    - Occurs as cell growth slows down
    - Solvent production such as butanol, ethanol and acetone.

# Pathway for the reaction



# Efficiency of aerobic and anaerobic respiration

- **Aerobic respiration:**

During aerobic respiration, 38 mol of ATP are produced for every molecule of glucose that is oxidized

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 38\ ATP$$

The energy released by the complete oxidation of glucose is 2880 KJ per mol.

The energy obtained in one mole of ATP is 30.6 KJ.

Therefore, energy obtained in 38 moles of ATP is 30.6 X 38 = 1162.8 KJ.

Therefore, the efficiency of transfer of energy in aerobic respiration is 1162.8/ 2880 = 40.4%

- **Anaerobic respiration**

**1. Yeast** (alcoholic fermentation): During alcoholic fermentation, two molecules of ATP are produced for every molecule of glucose used.

$$\text{Glucose} \rightarrow 2 \text{ ethanol} + 2CO_2 + 2ATP$$

The total energy released by conversion of glucose to ethanol is 210 KJ per mole.

The energy obtained in two molecules of ATP is 2 X 30.6 KJ = 61.2 KJ

Therefore, efficiency of transfer of energy during alcoholic fermentation is 61.2/210 = 29.1%

**2. Muscle** (Lactate fermentation): During lactate fermentation, two molecules of ATP are produced for every molecule of glucose used.

$$\text{Glucose} \rightarrow 2 \text{ lactate} + 2ATP$$

The total energy released by conversion of glucose to lactate is 150 KJ per mole.

Therefore, efficiency of transfer of energy during lactate fermentation is 61.2/150 = 40.8%

This indicates that the efficiency of each system is relatively high when compared with petrol engines (25-30%) and steam engines (8-12%)

The amount of energy captured as ATP during aerobic respiration is 19 times as much as for anaerobic respiration (38 ATP compared to 2 ATP).

From this point of view, aerobic respiration is much more efficient than anaerobic respiration. This is because a great deal of energy remains locked within ethanol and lactate.

The energy in ethanol is permanently unavailable to yeast, which clearly indicates that alcoholic fermentation is an inefficient energy producing process. However, much of the energy locked in lactate may be liberated at a later stage if oxygen is made available.

In the presence of oxygen, lactate is converted to pyruvate in liver. Pyruvate then enters Kreb's cycle and is fully oxidized to $CO_2$ and $H_2O$, releasing many more ATP molecules in the process. Alternatively, the pyruvate can be converted back to glucose by the reverse of glycolysis (gluconeogenesis) using energy from ATP

Based on the type of fermentation process, lactic acid fermenters can be divided into two groups.

## Homolactic / homofermentative bacteria

Include most of the species of *Lactobacillus*, *Streptococcus*, *Pediococcus*. They carry out a simple fermentation in which **lactic acid** is the only product formed.

Glucose → 2 Lactic acid.

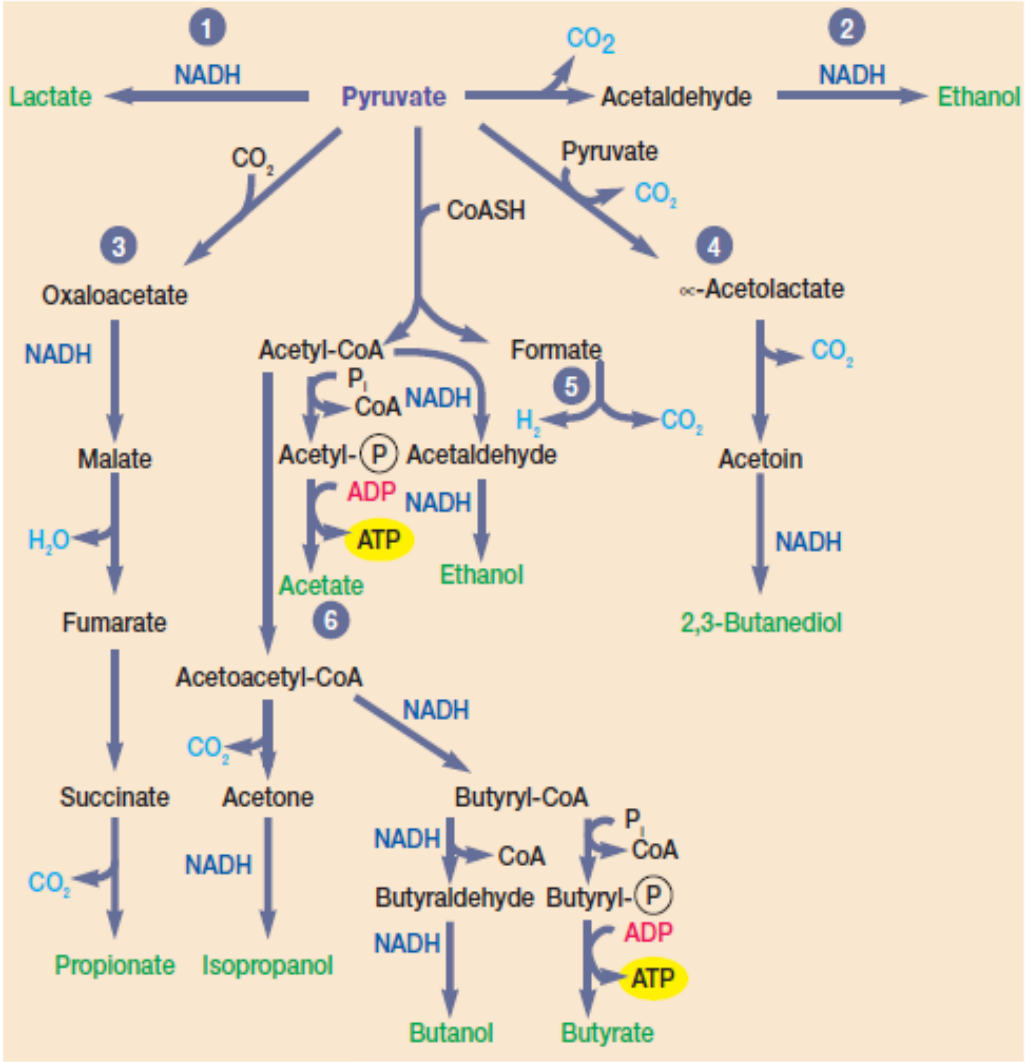
the **EMP pathway** is used in this process. The pyruvic acid formed is reduced by the enzyme lactate dehydrogenase to lactic acid. **2 ATPs** are generated for one glucose molecule.

## Heterolactic / heterofermentative bacteria

e.g. *Lactobacillus brevis*, *L. fermentum*, *Leuconostoc*. These bacteria produce **ethanol** and **$CO_2$** along with the **lactic acid**

Glucose → Lactic acid + Ethanol + $CO_2$

The heterofermentative bacteria do not use glycolysis, but use a **phosphoketolase pathway** for fermentation. Only **one ATP** is produced per molecule of glucose.




1. Lactic acid bacteria (*Streptococcus, Lactobacillus*), *Bacillus*
2. Yeast, *Zymomonas*
3. Propionic acid bacteria (*Propionibacterium*)
4. *Enterobacter, Serratia, Bacillus*
5. Enteric bacteria (*Escherichia, Enterobacter, Salmonella, Proteus*)
6. *Clostridium*

Some Common Microbial Fermentations


Thank
you!