MICROBIOLOGY MBT 202

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Microbial Metabolisms

All microbial metabolisms can be arranged according to three principles:

1. How the organism obtains carbon for synthesizing cell mass?

- • **autotrophic** – carbon is obtained from carbon dioxide (CO_2)

  • **heterotrophic** – carbon is obtained from organic compounds

  • **mixotrophic** – carbon is obtained from both organic compounds and by fixing carbon dioxide

2. How the organism obtains reducing equivalents used either in energy conservation or in biosynthetic reactions:

- **lithotrophic** – reducing equivalents are obtained from inorganic compounds

  organotrophic – reducing equivalents are obtained from organic compounds

3. How the organism obtains energy for living and growing:

- • **chemotrophic** – energy is obtained from external chemical compounds

  • **phototrophic** – energy is obtained from light.

  • **chemolithoautotrophs** obtain energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide. Examples: Nitrifying bacteria, Sulfur-oxidizing bacteria, Iron-oxidizing bacteria, Knallgas-bacteria

  • **photolithoautotrophs** obtain energy from light and carbon from the fixation of carbon dioxide, using reducing equivalents from inorganic compounds. Examples: Cyanobacteria (water (H_2O) as reducing equivalent donor), Chlorobiaceae, Chromatiaceae (hydrogen sulfide (H_2S) as reducing equivalent donor), *Chloroflexus* (hydrogen (H_2) as reducing equivalent donor)

  • **chemolithoheterotrophs** obtain energy from the oxidation of inorganic compounds, but cannot fix carbon dioxide (CO_2). Examples: some *Thiobacilus*, some *Beggiatoa*, some *Nitrobacter* spp., *Wolinella* (with H_2 as reducing equivalent donor), some Knallgas-bacteria, some sulfate-reducing bacteria

  • **chemoorganoheterotrophs** obtain energy, carbon, and reducing equivalents for biosynthetic reactions from organic compounds. Examples: most bacteria, e. g. *Escherichia coli*, *Bacillus* spp., *Actinobacteria*

• **photoorganoheterotrophs** obtain energy from light, carbon and reducing equivalents for biosynthetic reactions from organic compounds. Some species are strictly heterotrophic, many others can also fix carbon dioxide and are mixotrophic. Examples: *Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Rhodomicrobium*, *Rhodocy clus*, *Heliobacterium*, *Chloroflexus* (alternatively to photolithoautotrophy with hydrogen).

Diversity of electron acceptors for respiration

- **Organic compounds**:
  - Eg. fumarate, dimethylsulfoxide (DMSO), Trimethylamine-N-oxide (TMAO)
- **Inorganic compounds**:
  - Eg. NO_3^-, NO_2^-, SO_4^{2-}, S^0, SeO_4^{2-}, AsO_4^{3-}
- **Metals**:
  - Eg. Fe^{3+}, Mn^{4+}, Cr^{6+}
- **Minerals/solids**:
  - Eg. $Fe(OH)_3$, MnO_2
- **Gasses**:
  - Eg. NO, N_2O, CO_2

Most microorganisms oxidize carbohydrates as their main source of cellular energy.Microorganisms use two general processes: Cellular respiration and fermentation. Microorganisms also use anaerobic pathway to oxidize glucose. In case of aerobic respiration, the ultimate e-acceptor is O_2 and the reduced form is H_2O. There are f our stages of aerobic respiration:

Oxygen extracts chemical energy from glucose, the glucose molecule must be split up into two molecules of pyruvate. This process also generates two molecules of adenosine triphosphate as an immediate energy yield and two molecules of NADH.

- • The citric acid cycle begins with the transfer of a two-carbon acetyl group from acetyl-CoA to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).

  • The citrate then goes through a series of chemical transformations, losing two carboxyl groups as CO_2. The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA. The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle. Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric acid cycle in anabolism, they may not be lost, since many TCA cycle intermediates are also used as precursors for the biosynthesis of other molecules.

• Most of the energy made available by the oxidative steps of the cycle is transferred as energy-rich electrons to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced.

• Electrons are also transferred to the electron acceptor Q, forming QH_2.

• At the end of each cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

Anaerobic respiration - Microbes are capable of using all sorts of other terminal electron accepters besides oxygen. A few examples of anaerobic respiration;

• Final electron acceptor is an inorganic substance other than O_2.

• Some bacteria such as *Pseudomonas* and *Bacillus* can use a nitrate ion (NO^-_3), in the presence of an enzyme called nitrate reductase, as a final electron acceptor, the nitrate ion is reduced to nitrite ion (NO_2-).

• Nitrite ion can be converted to nitrous oxide (N_2O), or nitrogen gas (N_2) (denitrification process) which helps in recycling of nitrogen.

• Other bacteria like *Desulfovibrio* use sulfate (SO_4^{2-}) as the final electron acceptor and forms hydrogen sulfide (H_2S).

• Still other bacteria use carbonate (CO_3^{2-}) to form methane (CH_4).

• Anaerobic respiration by bacteria using nitrate and sulfate as final electron acceptors is essential for the nitrogen and sulfur cycles that occur in nature.

• Amount of ATP generated varies with the organisms and the pathway. Because only a part of the Krebs cycle operates and since not all the carriers in the electron transport chain participate, ATP yield is less and accordingly anaerobes tend to grow more slowly than aerobes.

Photosynthesis is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP. Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the "halobacteria" (actually archaea). The cyanobacteria conduct plant photosynthesis, called **oxygenic photosynthesis**; the purple and green bacteria conduct bacterial photosynthesis or **anoxygenic photosynthesis**; the extreme halophilic archaea use a type of **nonphotosynthetic photophosphorylation** mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.

Net equation:

$$\mathbf{6CO_2 + 12H_2O + LightEnergy \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_20}$$

Photosynthetic reactions divided into two stages:

- **Light reaction** - light energy absorbed & converted to chemical energy (ATP, NADPH)
- **Dark reaction-** carbohydrates made from CO_2 using energy stored in ATP & NADPH

Types of bacterial photosynthesis

Five photosynthetic groups within domain Bacteria (based on 16S rRNA):

1. Oxygenic Photosynthesis

- • Occurs in cyanobacteria and prochlorophytes

  • Synthesis of carbohydrates results in release of molecular O_2 and removal of CO_2 from atmoshphere.

  • Occurs in lamellae which house thylakoids containing chlorophyll a/b and phycobilisomes pigments which gather light energy

  • Uses two photosystems (PS):

    - PS II- generates a proton-motive force for making ATP.

    - PS I- generates low potential electrons for reducing power.

2. Anoxygenic Photosynthesis

- • Uses light energy to create organic compounds, and sulfur or fumarate compounds instead of O_2.

  • Occurs in purple bacteria, green sulfur bacteria, green gliding bacteria and heliobacteria.

  • Uses bacteriochlorophyll pigments instead of chlorophyll.

  • Uses one photosystem (PS I) to generate ATP in "cyclic" manner.

Light Reaction

The Light Reactions depend upon the presence of chlorophyll, the **primary light-harvesting pigment** in the membrane of photosynthetic organisms. The functional components of the photochemical system are **light harvesting pigments**, a membrane **electron transport system**, and an **ATPase** enzyme. The photosynthetic electron transport system of is fundamentally similar to a respiratory ETS, except that there is a low redox electron acceptor (e.g. **ferredoxin**) at the top (low redox end) of the electron transport chain, that is first reduced by the electron displaced from chlorophyll.

There are several types of pigments distributed among various phototrophic organisms. **Chlorophyll** is the primary light-harvesting pigment in all photosynthetic organisms. Chlorophyll is a tetrapyrrole which contains magnesium at the center of the porphyrin ring. It contains a long hydrophobic side chain that associates with the photosynthetic membrane. Cyanobacteria have **chlorophyll a**, the same as plants and algae. The chlorophylls of the purple and green bacteria, called **bacteriochlorophylls** are chemically different than chlorophyll a in their substituent side chains. This is reflected in their light absorption spectra. Chlorophyll a absorbs light in two regions of the spectrum, one around 450nm and the other between 650 - 750nm; bacterial chlorophylls absorb from 800-1000nm in the far red region of the spectrum.

Carotenoids are always associated with the photosynthetic apparatus. They function as **secondary light-harvesting pigments** , absorbing light in the blue-green spectral region

between 400-550 nm. Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wave lengths of light that are missed by chlorophyll. In addition, carotenoids have an indispensable function to protect the photosynthetic apparatus from photooxidative damage. Carotenoids have long hydrocarbon side chains in a conjugated double bond system. Carotenoids "quench" the powerful oxygen radical, singlet oxygen, which is invariably produced in reactions between chlorophyll and O_2 (molecular oxygen). Some non-photosynthetic bacterial pathogens, i.e., *Staphylococcus aureus,* produce carotenoids that protect the cells from lethal oxidations by singlet oxygen in phagocytes.

Phycobiliproteins are the major light harvesting pigments of the cyanobacteria. They also occur in some groups of algae. They may be red or blue, absorbing light in the middle of the spectrum between 550 and 650nm. Phycobiliproteins consist of proteins that contain covalently-bound linear tetrapyrroles (**phycobilins**). They are contained in granules called **phycobilisomes** that are closely associated with the photosynthetic apparatus. Being closely linked to chlorophyll they can efficiently transfer light energy to chlorophyll at the reaction center.

All phototrophic bacteria are capable of performing cyclic photophosphorylation as described above and in Figure 16 and below in Figure 18. This universal mechanism of cyclic photophosphorylation is referred to as **Photosystem I**. Bacterial photosynthesis uses only Photosystem I (PSI), but the more evolved cyanobacteria, as well as algae and plants, have an additional light-harvesting system called Photosystem II (PSII). **Photosystem II** is used to reduce Photosystem I when electrons are withdrawn from PSI for CO_2 fixation. PSII transfers electrons from H_2O and produces O_2, as shown in Figure 20.

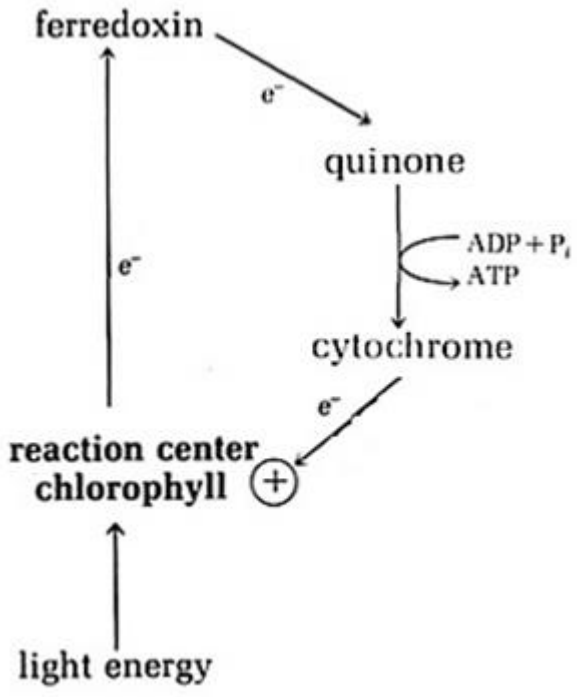


Fig. 15. The cyclical flow of electrons during anoxygenic photosynthesis.

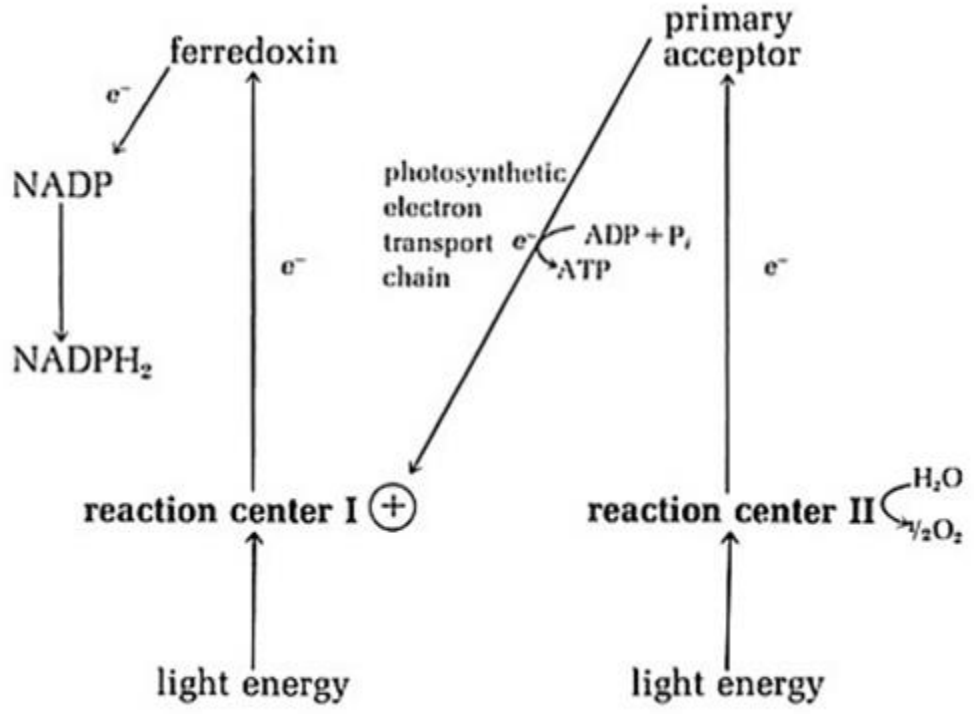


Fig. 16. Electron flow in oxygenic photosynthesis.

Dark reaction

The use of **RUBP carboxylase and the Calvin cycle** is the most common mechanism for CO_2 fixation among autotrophs. Indeed, RUBP carboxylase is said to be the most abundant enzyme on the planet (nitrogenase, which fixes N_2 is second most abundant). This is the only mechanism of autotrophic CO_2 fixation among eucaryotes, and it is used, as well, by all cyanobacteria and purple bacteria. Lithoautotrophic bacteria also use this pathway. But the green bacteria and the methanogens, as well as a few isolated groups of procaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase.

RUBP carboxylase (**ribulose bisphosphate carboxylase**) uses ribulose bisphosphate (RUBP) and CO_2 as co-substrates. In a complicated reaction the CO_2 is "fixed" by addition to the RUBP, which is immediately cleaved into two molecules of 3-phosphoglyceric acid (PGA). The fixed CO_2 winds up in the -COO group of one of the PGA molecules. Actually, this is the reaction which initiates the Calvin cycle (Fig. 3).

The Calvin cycle is concerned with the conversion of PGA to intermediates in glycolysis that can be used for biosynthesis, and with the regeneration of RUBP, the substrate that drives the cycle. After the initial fixation of CO_2, 2 PGA are reduced and combined to form hexose-phosphate by reactions which are essentially the reverse of the oxidative Embden-Meyerhof pathway. The hexose phosphate is converted to pentose-phosphate, which is phosphorylated to regenerate RUBP. An important function of the Calvin cycle is to provide the organic precursors for the biosynthesis of cell material. Intermediates must be constantly withdrawn from the Calvin cycle in order to make cell material. In this regard, the Calvin cycle is an anabolic pathway. The fixation of CO_2 to the level of glucose ($C_6H_{12}O_6$) requires 18 ATP and 12 $NADPH_2$.

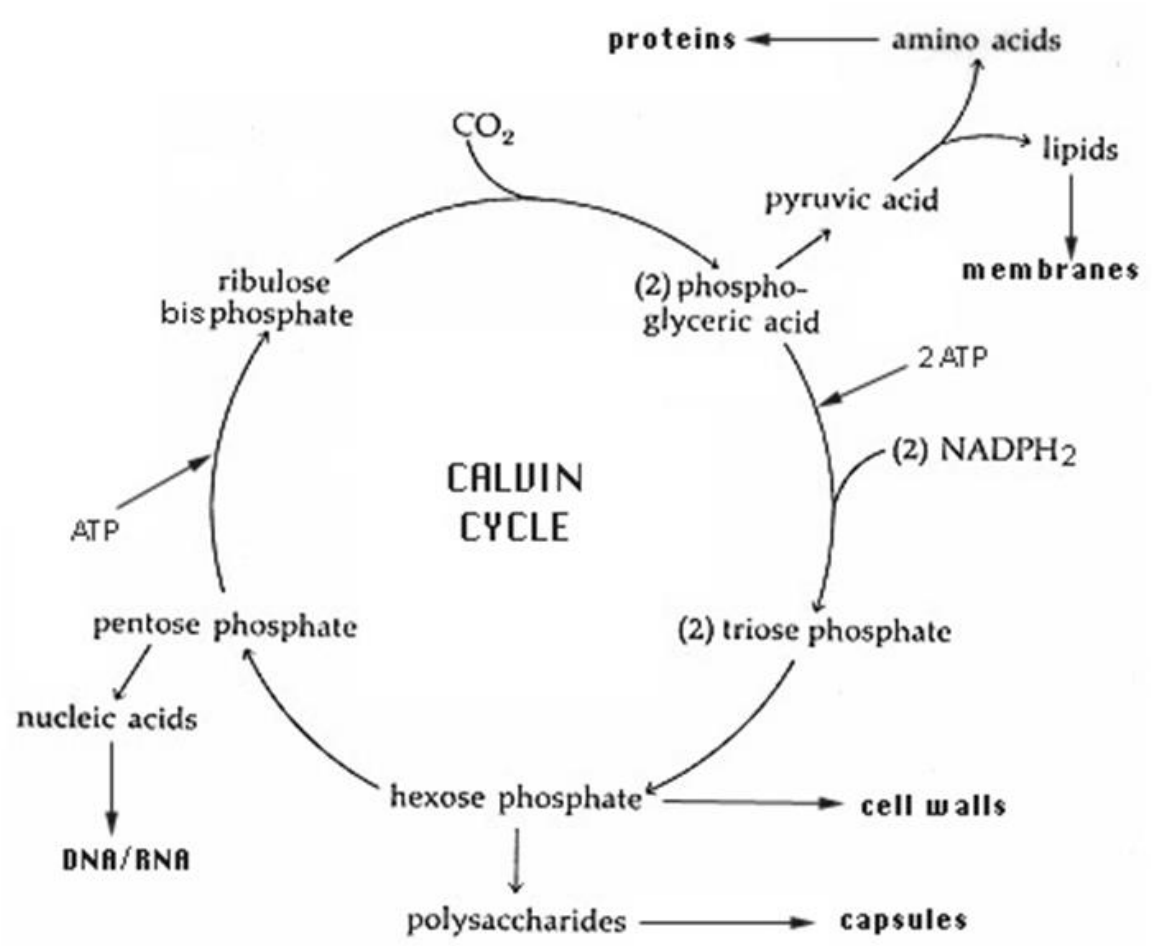


Fig. 17. The Calvin cycle and its relationship to the synthesis of cell materials.

Most of the phototrophic procaryotes are autotrophs, which mean that they are able to fix CO_2 as a sole source of carbon for growth. Just as the oxidation of organic material yields energy, electrons and CO_2, in order to build up CO_2 to the level of cell material (CH_2O), energy (ATP) and electrons (reducing power) are required. The overall reaction for the fixation of CO_2 in the Calvin cycle is **CO_2 + 3ATP + 2$NADPH_2$ ----------> CH_2O + 2ADP + 2Pi + 2NADP**. The light reactions operate to produce ATP to provide energy for the dark reactions of CO_2 fixation. The dark reactions also need reductant (electrons). Usually the provision of electrons is in some way connected to the light reactions.

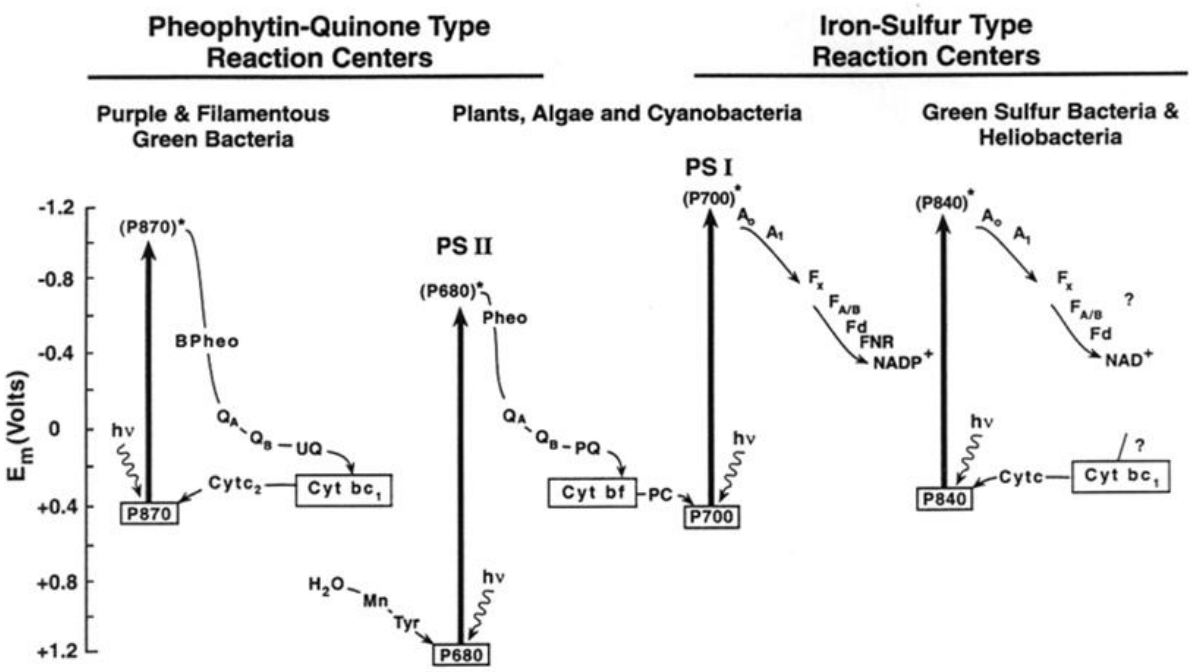
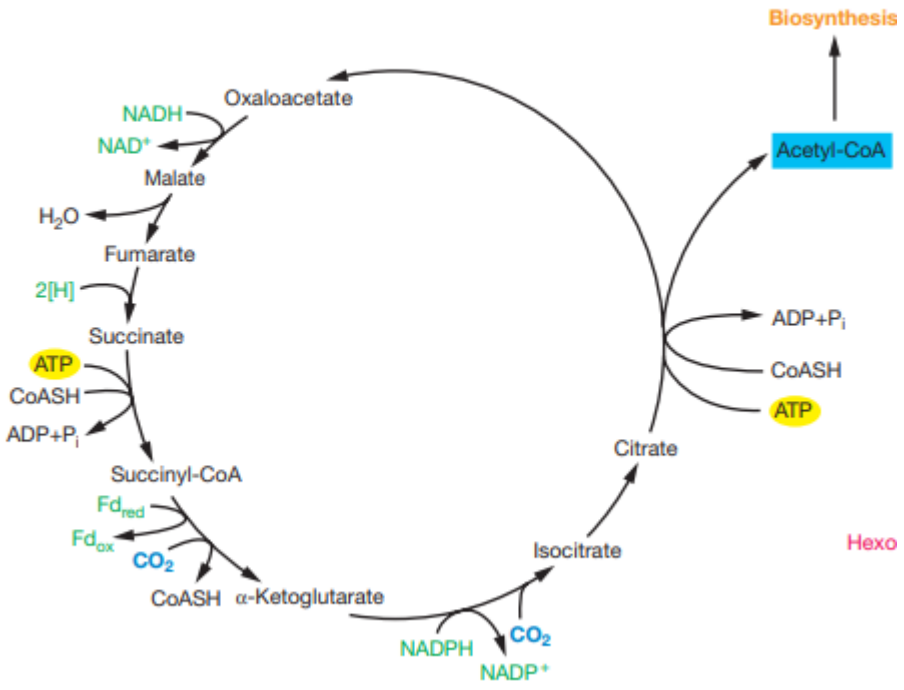


Fig. 18. Comparison of electron transport pathways in oxygenic and anoxygenic photosynthesis

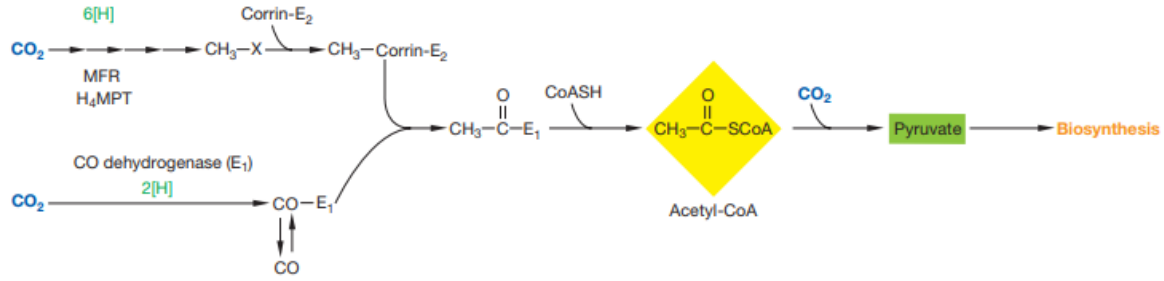
The differences between plant and bacterial photosynthesis are summarized in Table 3 below. Bacterial photosynthesis is an anoxygenic process. The external electron donor for bacterial photosynthesis is never H_2O, and therefore, purple and green bacteria never produce O_2 during photosynthesis. Furthermore, bacterial photosynthesis is usually inhibited by O_2 and takes place in microaerophilic and anaerobic environments. Bacterial chlorophylls use light at longer wave lengths not utilized in plant photosynthesis, and therefore they do not have to compete with oxygenic phototrophs for light. Bacteria use only cyclic photophosphorylation (Photosystem I) for ATP synthesis and lack a second photosystem.

Table 3. Differences between plant and bacterial photosynthesis

	Plant photosynthesis	Bacterial photosynthesis
Organisms	**Plants, algae, cyanobacteria**	**Purple and green bacteria**
Type of chlorophyll	Chlorophyll-a and absorbs 650-750nm	bacteriochlorophyll and absorbs 800-1000nm
Photosystem I (cyclic photophosphorylation)	present	present
Photosystem I (noncyclic photophosphorylation)	present	absent
Produces O_2	yes	no
Photosynthetic electron donor	H_2O	H_2S, other sulfur compounds or certain organic compounds



Reductive TCA cycle



The Reductive Acetyl-CoA Pathway. Methanogens reduce two molecules of CO2, each by a different mechanism, and combine them to form acetyl and then acetyl-CoA.

Comparison of Archaeal CO_2-Fixation Pathways to the Calvin-Benson Cycle

CO_2-Fixation Pathway	Oxygen Tolerance	ATP Consumed per Pyruvate Produced	Archaeal Phyla Known to Use Pathway
Reductive Acetyl-CoA	Anaerobic	1	*Euryarchaeota*
3-Hydroxypropionate/ 4-Hydroxybutyrate	Aerobic	9	*Crenarchaeota* *Thaumarchaeota*
Dicarboxylate/ 4-Hydroxybutyrate	Anaerobic	5	*Crenarchaeota*
Calvin-Benson	Aerobic	7	None

Chemosynthesis

Chemosynthesis is the biological conversion of one or more carbon molecules (usually carbon dioxide or methane) and nutrients into organic matter using the oxidation of inorganic molecules (e.g. hydrogen gas, hydrogen sulfide) or methane as a source of energy, rather than sunlight, as in photosynthesis. but groups that include conspicuous or biogeochemically-important taxa include the sulfur-oxidizing gamma and epsilon proteobacteria, the Aquificaeles, the Methanogenic archaea and the neutrophilic iron-oxidizing bacteria.

Chemoautotrophs or lithotrophs, organisms that obtain carbon through chemosynthesis, are phylogenetically diverse, united only by their ability to oxidize an inorganic compound as an energy source. Chemosynthesis runs through the **Bacteria** and the **Archaea**. Chemoautotrophs are usually organized into "physiological groups" based on their inorganic substrate for energy production and growth (see Table 2 below).

T able 2. Physiological groups of chemoautotrophs

Physiological group	Energy source	Oxidized end product	Organism
Hydrogen bacteria	H_2	H_2O	*Alcaligenes, Pseudomonas*
Methanogens	H_2	H_2O	*Methanobacterium*
Carboxydobacteria	CO	CO_2	*Rhodospirillum, Azotobacter*
Nitrifying bacteria*	NH_3	NO_2	*Nitrosomonas*
Nitrifying bacteria*	NO_2	NO_3	*Nitrobacter*
Sulfur oxidizers	H_2S or S	SO_4	*Thiobacillus, Sulfolobus*
Iron bacteria	Fe^{++}	Fe^{+++}	*Gallionella, Thiobacillus*

*The overall process of **nitrification**, conversion of NH_3 to NO_3, requires a consortium of microorganisms.

The **hydrogen bacteria** oxidize H_2 (hydrogen gas) as an energy source. The hydrogen bacteria are **facultative lithotrophs** as evidenced by the pseudomonads that fortuitously possess a hydrogenase enzyme that will oxidize H_2 and put the electrons into their respiratory ETS. They will use H_2 if they find it in their environment even though they are typically heterotrophic. Indeed, most hydrogen bacteria are nutritionally versatile in their ability to use a wide range of carbon and energy sources.

The **methanogens** used to be considered a major group of hydrogen bacteria - until it was discovered that they are **Archaea**. The methanogens are able to oxidize H_2 as a sole source of energy while transferring the electrons from H_2 to CO_2 in its reduction to methane. Metabolism of the methanogens is absolutely unique, yet methanogens represent the most prevalent and diverse group of **Archaea**. Methanogens use H_2 and CO_2 to produce cell material and methane. They have unique enzymes and electron transport processes. Their type of energy generating metabolism is never seen in the **Bacteria**, and their mechanism of autotrophic CO_2 fixation is very rare, except in methanogens.

The **carboxydobacteria** are able to oxidize CO (carbon monoxide) to CO_2, using an enzyme **CODH** (**carbon monoxide dehydrogenase**). The carboxydobacteria are not obligate CO users, i.e., some are also hydrogen bacteria, and some are phototrophic bacteria. Interestingly, the enzyme CODH used by the carboxydobacteria to oxidize CO to CO_2, is used by the methanogens for theThe **nitrifying bacteria** are represented by two genera, *Nitrosomonas* and *Nitrobacter.* Together these bacteria can accomplish the oxidation of NH_3 to NO_3, known as the process of **nitrification**. No single organism can carry out the whole oxidative process. *Nitrosomonas* oxidizes ammonia to NO_2 and *Nitrobacter* oxidizes NO_2 to NO_3. Most of the nitrifying bacteria are **obligate lithoautotrophs**, the exception being a few strains of *Nitrobacter* that will utilize acetate. CO_2 fixation utilizes RUBP carboxylase and the Calvin Cycle. Nitrifying bacteria grow in environments rich in ammonia, where extensive protein decomposition is taking place. Nitrification in soil and aquatic habitats is an essential part of the nitrogen cycle.

Chemoautotrophic **sulfur oxidizers** include both **Bacteria** (e.g. *Thiobacillus*) and **Archaea** (e.g. *Sulfolobus*). Sulfur oxidizers oxidize H_2S (sulfide) or S (elemental sulfur) as a source of energy. Similarly, the purple and green sulfur bacteria oxidize H_2S or S as an electron donor for photosynthesis, and use the electrons for CO_2 fixation (the dark reaction of photosynthesis). Obligate autotrophy, which is nearly universal among the nitrifiers, is variable among the sulfur oxidizers. Lithoautotrophic sulfur oxidizers are found in environments rich in H_2S,

such as volcanic hot springs and fumaroles, and deep-sea thermal vents. Some are found as symbionts and endosymbionts of higher organisms. Since they can generate energy from an inorganic compound and fix CO_2 as autotrophs, they may play a fundamental role in **primary production** in environments that lack sunlight. As a result of their lithotrophic oxidations, these organisms produce sulfuric acid (SO_4), and therefore tend to acidify their own environments. Some of the sulfur oxidizers are **acidophiles** that will grow at a pH of 1 or less. Some are **hyperthermophiles** that grow at temperatures of 115°C.

Iron bacteria oxidize Fe^{++} (ferrous iron) to Fe^{+++} (ferric iron). At least two bacteria probably oxidize Fe^{++} as a source of energy and/or electrons and are capable of chemoautotrophic growth: the stalked bacterium *Gallionella*, which forms flocculant rust-colored colonies attached to objects in nature, and *Thiobacillus ferrooxidans*, which is also a sulfur-oxidizing lithotroph.

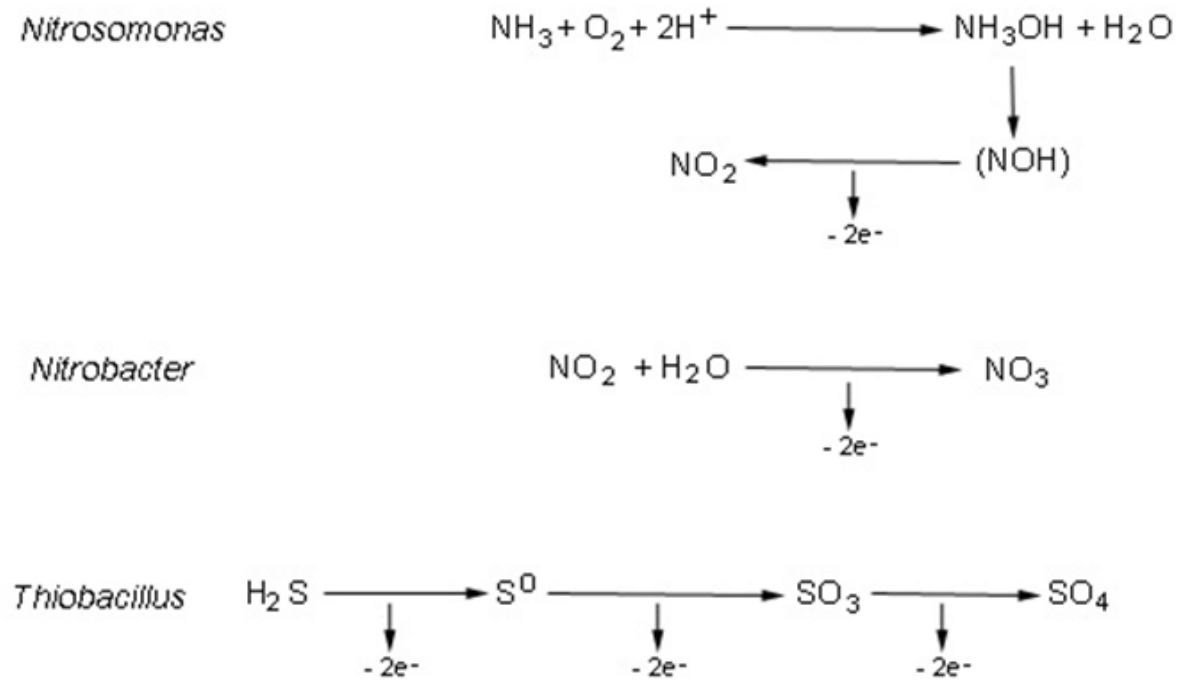


Fig. 19. Chemoautotrophic or Lithotrophic oxidations. These reactions produce energy for metabolism in the nitrifying and sulfur oxidizing bacteria.

reverse reaction - the reduction of CO_2 to CO - in their unique pathway of CO_2 fixation.

Anaerobic Respiration

Respiration in some prokaryotes is possible using electron acceptors other than oxygen (O_2). This type of respiration in the absence of oxygen is referred to as anaerobic respiration. Electron acceptors used by prokaryotes for respiration or methanogenesis (an analogous type of energy generation in archaea bacteria) are described in the table below.\

Terminal e-Acceptor	Reduced End Product	Process	Example
O_2	H_2O	aerobic respiration	*Escherichia, Streptomyces*
NO_3	NO_2, NH_3 or N_2	anaerobic respiration: denitrification	*Bacillus, Pseudomonas*
SO_4	S or H_2S	anaerobic respiration: sulfate reduction	*Desulfovibrio*
fumarate	succinate	anaerobic respiration: using an organic e-acceptor	*Escherichia*
CO_2	CH_4	Methanogenesis	*Methanococcus*

Biological methanogenesis is the source of methane (natural gas) on the planet. Methane is preserved as a fossil fuel (until we use it all up) because it is produced and stored under anaerobic conditions, and oxygen is needed to oxidize the CH_4 molecule.

Methanogenesis is not really a form of anaerobic respiration, but it is a type of energy-generating metabolism that requires an outside electron acceptor in the form of CO_2.

Sulfate reduction is not an alternative to the use of O_2 as an electron acceptor. It is an obligatory process that occurs only under anaerobic conditions. Methanogens and sulfate reducers may share habitat, especially in the anaerobic sediments of eutrophic lakes such as Lake Mendota, where they crank out methane and hydrogen sulfide at a surprising rate.

Nitrate reduction

Some microbes are capable of using nitrate as their terminal electron accepter. The ETS used is somewhat similar to aerobic respiration, but the terminal electron transport protein donates its electrons to nitrate instead of oxygen. Nitrate reduction in some species (the best studied being *E. coli*) is a two electron transfer where nitrate is reduced to nitrite. Electrons flow through the quinone pool and the cytochrome b/c_1 complex and then nitrate reductase resulting in the transport of protons across the membrane as discussed earlier for aerobic respiration.

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

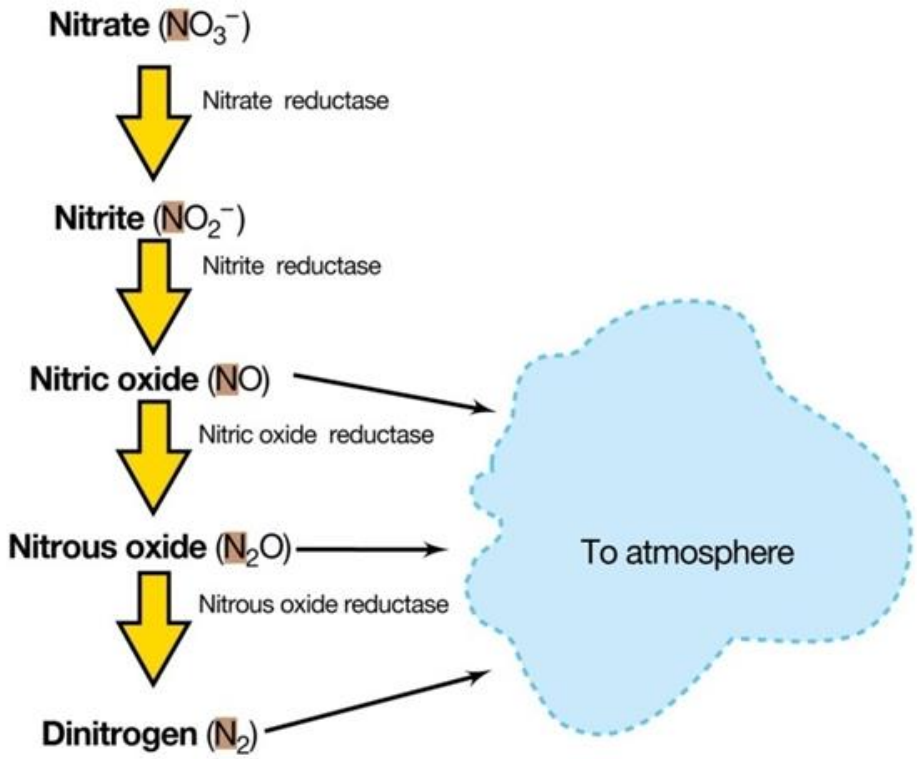


Fig. 7. Nitrate reduction

Steps in the dissimilative reduction of nitrate . Some organisms, for example *Escherichia coli* , can carry out only the first step. All enzymes involved are derepressed by anoxic conditions. Also, some prokaryotes are known that can reduce NO_3^- to NH_4^+ in dissimilative metabolism.

Denitrification

Denitrification is an important process in agriculture because it removes NO_3 from the soil. NO_3 is a major source of nitrogen fertilizer in agriculture. Almost one-third the cost of some types of agriculture is in nitrate fertilizers. The use of nitrate as a respiratory electron acceptor is usually an alternative to the use of oxygen. Therefore, soil bacteria such as *Pseudomonas* and *Bacillus* will use O_2 as an electron acceptor if it is available, and disregard NO_3. This is the rationale in maintaining well-aerated soils by the agricultural practices of plowing and tilling. *E. coli* will utilize NO_3 (as well as fumarate) as a respiratory electron acceptor and so it may be able to continue to respire in the anaerobic intestinal habitat.

Nitrite, the product of nitrate reduction, is still a highly oxidized molecule and can accept up to six more electrons before being fully reduced to nitrogen gas. Microbes exist (*Paracoccus species, Pseudomonas stutzeri, Pseudomonas aeruginosa* , and *Rhodobacter sphaeroides* are a few examples) that are able to reduce nitrate all the way to nitrogen gas. The process is carefully regulated by the microbe since some of the products of the reduction of nitrate to nitrogen gas are toxic to metabolism. This may explain the large number of genes involved in the process and the limited number of bacteria that are capable of denitrification. Below is the chemical equation for the reduction of nitrate to N_2.

$N0_3^- \longrightarrow N0_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$

Denitrification takes eight electrons from metabolism and adds them to nitrate to form N_2

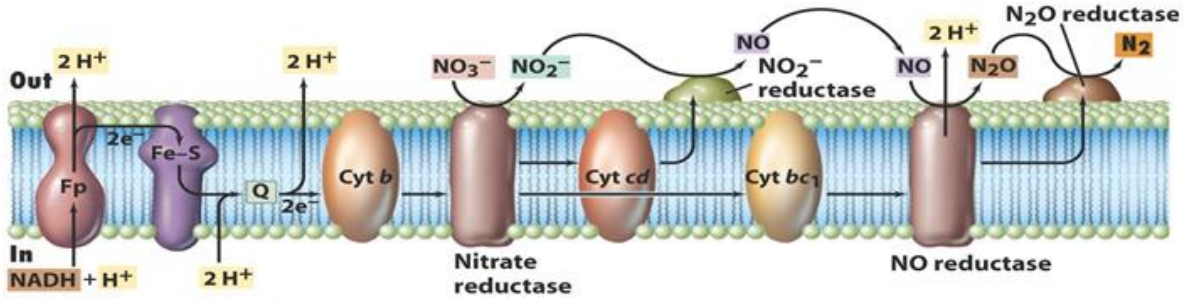


Fig. 8. Denitification by *Pseudomonas stutzeri*

- Four terminal reductases involved in denitrification steps;

- **Nar** : Nitrate reductase (Mo-containing enzyme)

- **Nir** : Nitrite reductase

- **Nor** : Nitric oxide reductase

- **N_2 Or** : Nitrous oxide reductase

- All can function independently but they operate in unison

Fermentation:

Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis.

During fermentation, **pyruvate is metabolised to various compounds**. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (a phenomenon known as the **Crabtree effect**).

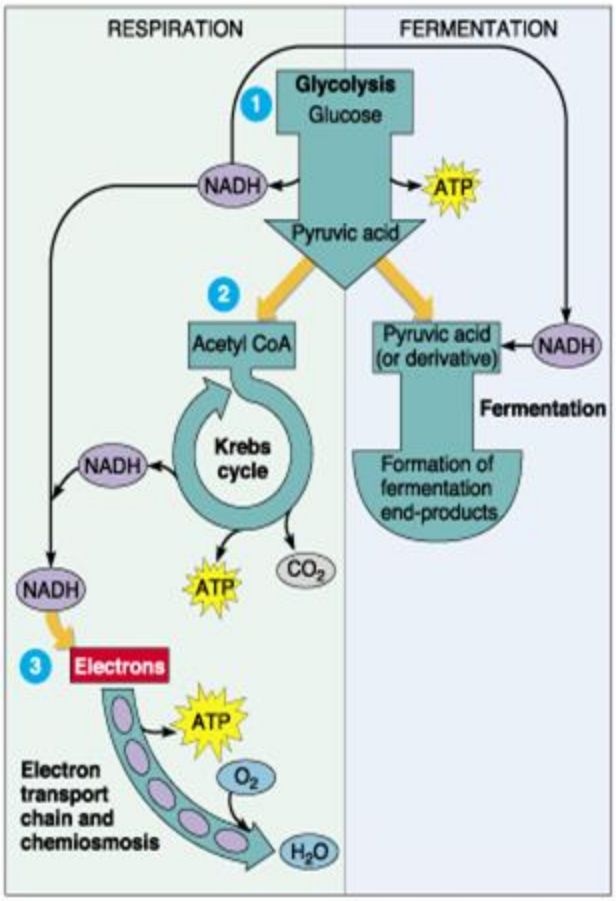


Fig. 9. Respiration and Fermentation pathways

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces – transfers electrons to – NAD+, forming NADH. However there is a limited supply of NAD+ available in any given cell.

- • For glycolysis to continue, NADH must be oxidized – have electrons taken away – to regenerate the NAD+ that is used in glycolysis. In an aerobic environment (Oxygen is available), reduction of NADH is usually done through an electron transport chain in a process called oxidative phosphorylation; however, oxidative phosphorylation cannot occur in anaerobic environments (Oxygen is not available) due to the pathways dependence on the terminal electron acceptor of oxygen.

  • Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate.

In heterolactic acid fermentation, one molecule of pyruvate is converted to lactate; the other is converted to ethanol and carbon dioxide.

Fermentation:

Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis.

During fermentation, **pyruvate is metabolised to various compounds**. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (a phenomenon known as the **Crabtree effect**).

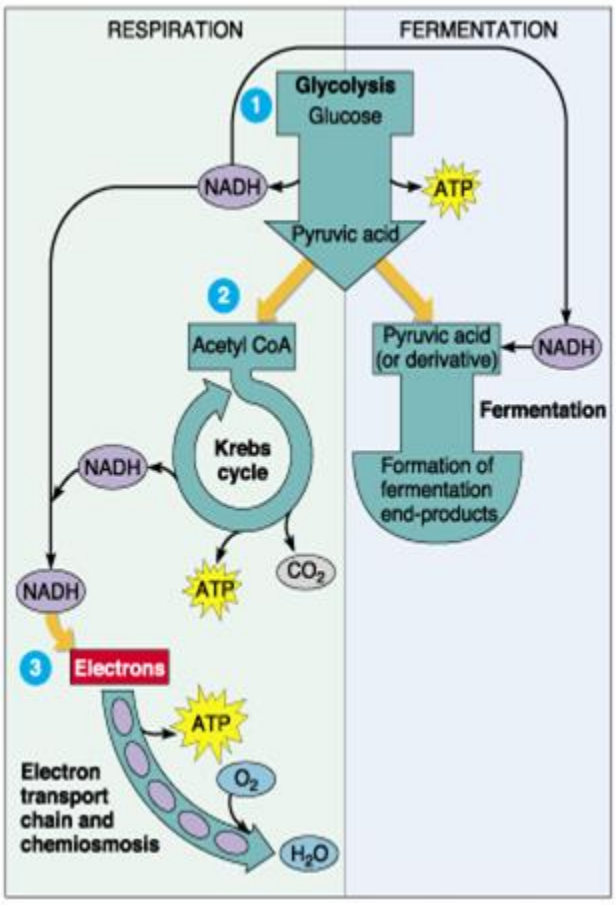


Fig. 9. Respiration and Fermentation pathways

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Mixed fermentations

Butanediol Fermentation. Forms mixed acids and gases as above, but, in addition, **2,3 butanediol** from the condensation of 2 pyruvate. The use of the pathway decreases acid formation (butanediol is neutral) and causes the formation of a distinctive intermediate, **acetoin**. Water microbiologists have specific tests to detect low acid and acetoin in order to distinguish non fecal enteric bacteria (butanediol formers, such as *Klebsiella* and *Enterobacter*) from fecal enterics (mixed acid fermenters, such as *E. coli, Salmonella* and *Shigella*).

Butyric acid fermentations, as well as the butanol-acetone fermentation (below), are run by the clostridia, the masters of fermentation. In addition to butyric acid, the clostridia form acetic acid, CO_2 and H_2 from the fermentation of sugars. Small amounts of ethanol and isopropanol may also be formed.

Butanol-acetone fermentation. Butanol and acetone were discovered as the main end products of fermentation by *Clostridium acetobutylicum* during the World War I. This discovery solved a critical problem of explosives manufacture (acetone is required in the manufacture gunpowder) and is said to have affected the outcome of the War. Acetone was distilled from the fermentation liquor of *Clostridium acetobutylicum,* which worked out pretty good if you were on our side, because organic chemists hadn't figured out how to synthesize it chemically. You can't run a war without gunpowder, at least you couldn't in those days.

Propionic acid fermentation . This is an unusual fermentation carried out by the propionic acid bacteria which include corynebacteria, *Propionibacterium* and *Bifidobacterium* . Although sugars can be fermented straight through to propionate, propionic acid bacteria will ferment lactate (the end product of lactic acid fermentation) to acetic acid, CO_2 and propionic acid. The formation of propionate is a complex and indirect process involving 5 or 6 reactions. Overall, 3 moles of lactate are converted to 2 moles of propionate + 1 mole of acetate + 1 mole of CO_2, and 1 mole of ATP is squeezed out in the process. The propionic acid bacteria are used in the manufacture of Swiss cheese, which is distinguished by the distinct flavor of propionate and acetate, and holes caused by entrapment of CO_2.

Microbial fermentation

Fermentation is a specific type of heterotrophic metabolism that uses organic carbon instead of oxygen as a terminal electron acceptor. This means that these organisms do not use an electron transport chain to oxidize NADH to NAD^+ and therefore must have an alternative method of using this reducing power and maintaining a supply of NAD^+ for the proper functioning of normal metabolic pathways (e.g. glycolysis). As oxygen is not required, fermentative organisms are anaerobic.

Many organisms can use fermentation under anaerobic conditions and aerobic respiration when oxygen is present. These organisms are **facultative anaerobes**. To avoid the overproduction of NADH, obligately fermentative organisms usually do not have a complete citric acid cycle. Instead of using an ATP synthase as in respiration, ATP in fermentative organisms is produced by **substrate-level phosphorylation** where a phosphate group is transferred from a high-energy organic compound to ADP to form ATP. As a result of the need to produce high energy phosphate-containing organic compounds (generally in the form of CoA -esters) fermentative organisms use NADH and other cofactors to produce many different reduced metabolic by-products, often including hydrogen gas (H_2). These reduced organic compounds are generally small organic acids and alcohols derived from pyruvate, the end product of glycolysis. Examples include ethanol, acetate, lactate, and butyrate. Fermentative organisms are very important industrially and are used to make many different types of food products. The different metabolic end products produced by each specific bacterial species are responsible for the different tastes and properties of each food.

The two main types of fermentation are alcoholic fermentation and lactic acid fermentation (Fig.2). The two main types of fermentation are:

1) **Alcoholic fermentation**

2) **Lactic acid fermentation**

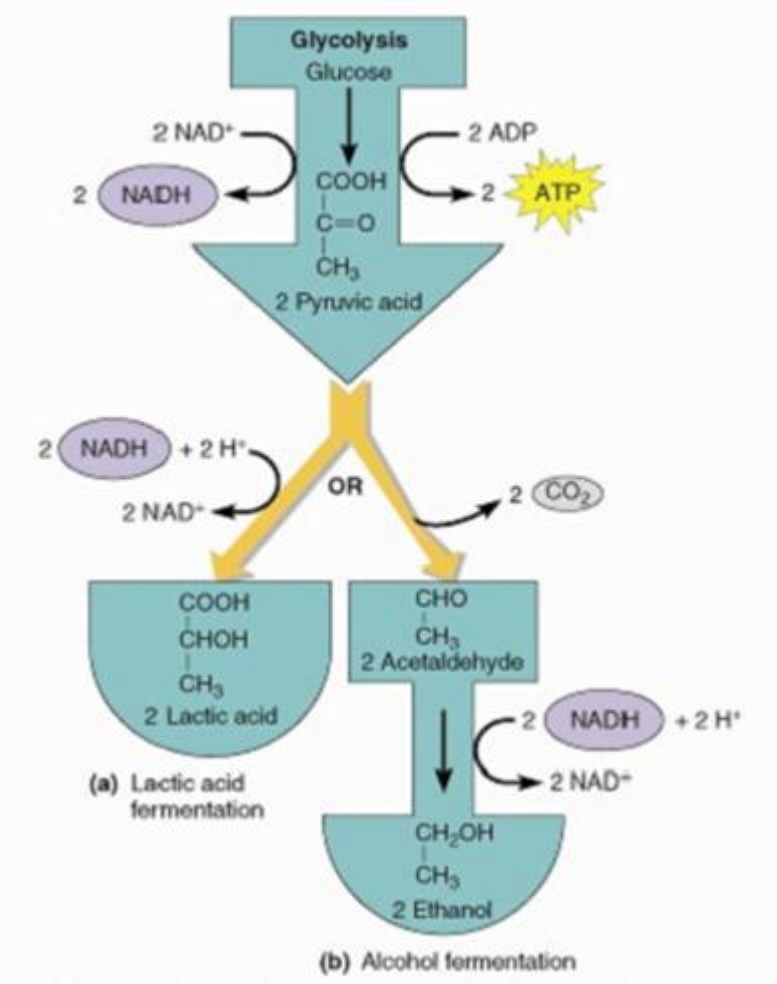


Fig. 2. Lactic acid and ethanolic fermentations

Both types have the same reactants: Pyruvic acid and NADH, both of which are products of glycolysis.

In alcoholic fermentation, the major products are alcohol and carbon dioxide. In lactic acid fermentation, the major product is lactic acid.

For both types of fermentation, there is a side product: NAD+ which is recycled back to glycolysis so that small amounts of ATP can continue to be produced in the absence of oxygen.

The chemical equations below summarize the fermentation of sucrose , whose chemical formula is $C_{12}H_{22}O_{11}$. One mole of sucrose is converted into four moles of ethanol and four moles of carbon dioxide :

$C_{12}H_{22}O_{11}+H_2O$ + invertase $\rightarrow 2C_6H_{12}O_6$

$C_6H_{12}O_6$+Zymase $\rightarrow 2C_2H_5OH + 2CO_2$

The process of lactic acid fermentation using glucose is summarized below. In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid:[3]

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$

In heterolactic fermentation, the reaction proceeds as follows, with one molecule of glucose converted to one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide:

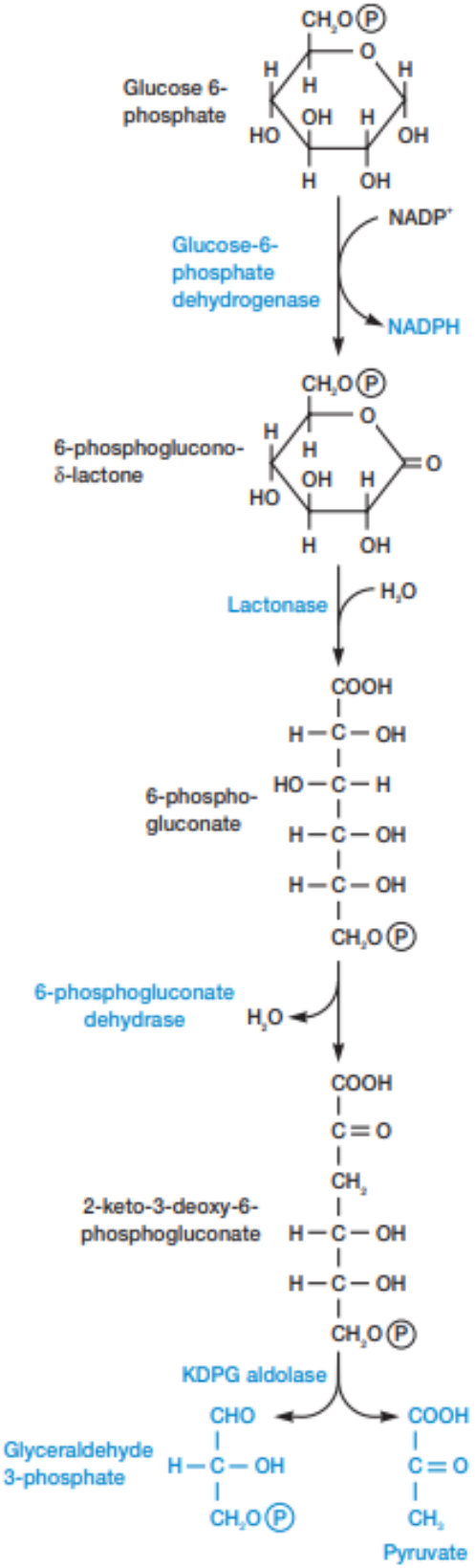
$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

Alternatives of Glycolysis:

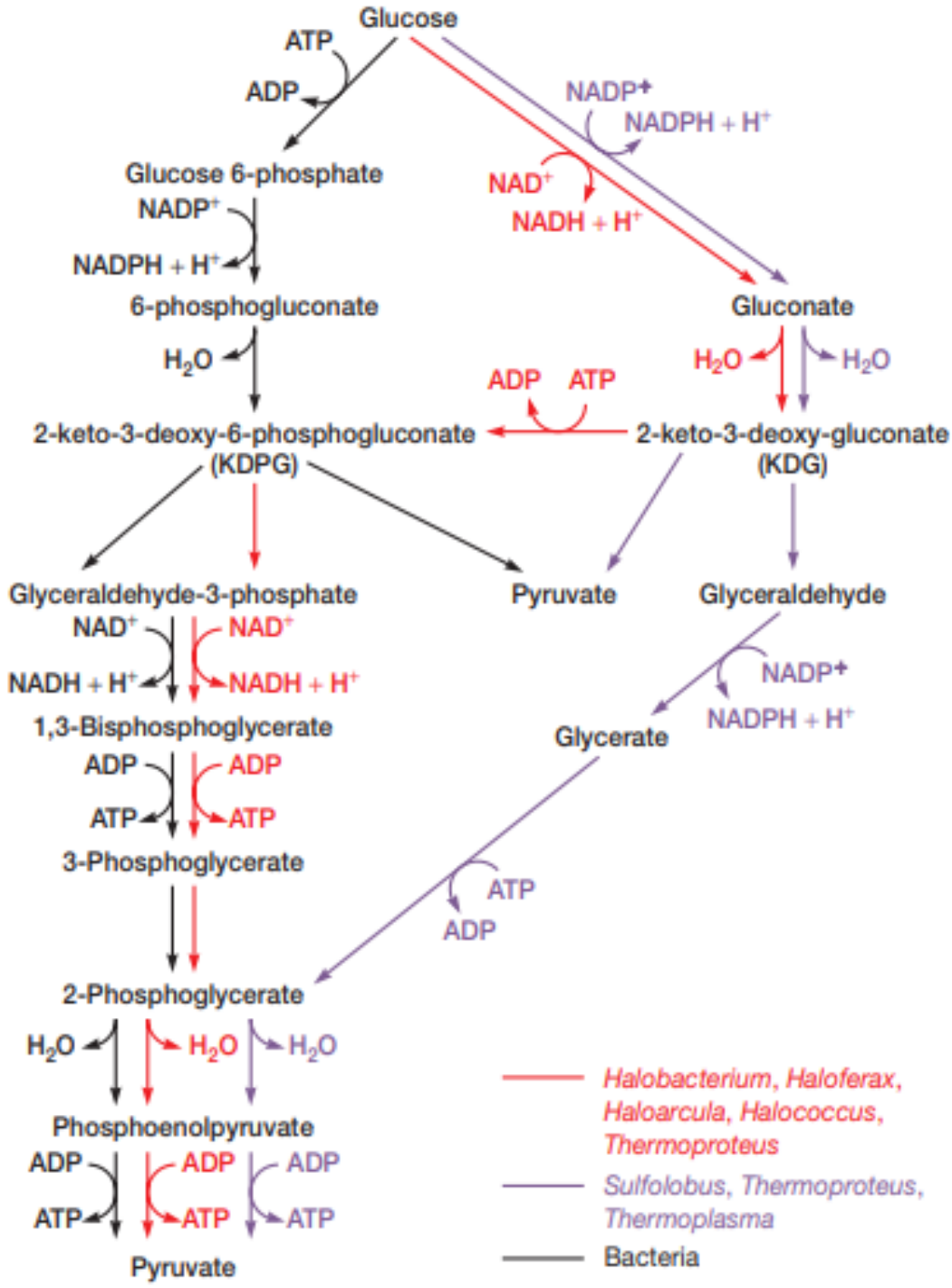
Many bacteria have another pathway in addition to glycolysis for the oxidation of glucose. The most common are i) pentose phosphate pathway and ii) Entner-Doudoroff pathway

1. Pentose Phosphate pathway (Hexose monophosphate shunt): This provides a means for the breakdown of five-carbon sugars (pentoses) as well as glucose. A key feature is that it produces important intermediates pentoses used in the synthesis of nucleic acids, glucose from Co_2 in photosynthesis and certain amino acids. The pathway is an important producer of the reduced coenzyme NADPH from $NADP^+$. This pathway yields a net gain of only **one** molecule of ATP for each molecule of glucose oxidised. Bacteria that use this pathway include *Bacillus subtilis, E.coli, Leuconostoc mesenteroides and Enterococcus faecalis.*

The Entner-Doudoroff pathway: For each molecule of glucose this pathway produces 2 molecules of NADPH and one molecule of ATP for use in cellular biosynthetic reactions. Bacteria that have the enzymes for this pathway can metabolize glucose without either glcolysis or the pentose phosphate pathway. Found in some gram-negative bacteria, including *Rhizobium* , *Pseudomonas* and *Agrobacterium* ; generally not found among gram-positive bacteria.



The Entner-Doudoroff Pathway.



Two Alternate Versions of the Entner-Doudoroff Pathway.

The phosphorylative pathway (red) used by halophilic archaea involves the ATPdependent phosphorylation of KDG to KDPG; the remainder of the pathway is like that of bacteria and an ATP is gained. The nonphosphorylative pathway (purple) used by Sulfolobus spp. and others joins the bacterial ED pathway when glycerate is converted to 2-phosphoglycerate, so no net ATP is produced

Cellular/Aerobic respiration

After glucose has been broken down to pyruvic acid, the pyruvic acid can be channeled into the next step of either fermentation or cellular respiration.

Cellular respiration – is defined as an ATP generating process in which molecules are oxidized and the final electron acceptor is an inorganic molecule. Two types of respiration occur, depending on whether an organism is an aerobe or an anaerobe. In aerobic respiration – the final electron acceptor is O_2 and in anaerobic respiration – it is an inorganic molecule other than O_2 or rarely an organic molecule.

The Krebs cycle /Citric Acid Cycle/ Tricarboxylic Acid Cycle

The pyruvate produced by glycolysis is oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and are not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways.

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion . Bacteria also use the TCA cycle to generate energy, but since they lack mitochondria, the reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the plasma membrane rather than the inner membrane of the mitochondrion.