MICROBIAL PHYSIOLOGY AND BIOCHEMISTRY

Carbon and nitrogen metabolism

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Keywords

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All living organisms require continuous supply of energy. This energy is utilized to carry out following major functions:

(i) Various cellular movements
(ii) The transport of molecules and ions
(iii) The synthesis of large molecules from simple precursor molecules (this occurs via biosynthetic pathways).

Different organisms obtain this energy from various sources like sunlight, oxidation of foodstuff, etc. The cells of the organism then carry out thousands of chemical reactions in order to extract energy from these sources. The sum total of these reactions in an organism is defined as metabolism.

The process of metabolism in the all organisms takes place via sequences of consecutive reactions catalyzed by enzymes. Each step is usually a single, highly specific, chemical change leading to a product that becomes the reactant for the next step. Such sequence of reactions that has a specific purpose is called as a metabolic pathway e.g. the degradation of glucose to pyruvate. A pathway may be linear (glycolysis), branched (biosynthesis of amino acids), cyclic (citric acid cycle), or spiral (fatty acid degradation).

**Overview of metabolism**

We can divide metabolic pathways into two classes:

1. **Catabolism** (Greek *cata* - down and *ballein* - to throw) includes those pathways in which larger and more complex molecules are broken down into smaller, simple molecules, e.g. molecules like fats, carbohydrates and proteins are degraded to simpler molecules such as lactate, pyruvate, ethanol, CO$_2$, water and ammonia. They result in release of energy. Some of this energy is stored and used for work while rest is released as heat.

   \[
   \text{Fuels (Carbohydrates, Fats)} \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{Useful energy}
   \]

2. **Anabolism** (Greek *ana*-up) includes the pathways involved in synthesis of large complex molecules from simpler ones e.g. the synthesis of proteins from amino acids, the formation of glucose from pyruvate molecules and the synthesis of DNA from nucleotides. Here, energy is required and is provided by catabolic processes.

   \[
   \text{Useful energy + Small molecules} \rightarrow \text{Complex molecules.}
   \]

Some pathways can be either anabolic or catabolic, depending on the energy conditions in the cell. They are called as amphibolic pathways.

**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. In other words, every cell stores and uses energy, biochemically, through ATP. Therefore, it is considered a universal currency of biological energy.
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 (Fig. 1) consisting of a purine 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.

![Fig. 1: The structure of adenosine triphosphate (ATP). ATP is a typical nucleotide consisting of an adenine ring, a ribose, and three phosphate groups.](Source: Concepts of Biochemistry; Rodney Boyer)

Energy released during various metabolic reactions gets stored in the covalent bonds between phosphate groups. The greatest amount of energy is stored between the second and third phosphate groups (from the ribose sugar). This covalent bond is known as a pyrophosphate/high energy bond. The amount of energy in ATP is written in terms of standard free energy change, $\Delta G^0$. Following changes occur when ATP gets hydrolyzed:

$$\text{Anabolism} \quad \text{ATP} + \text{H}_2\text{O} \quad \rightarrow \quad \text{ADP} + \text{Pi} \quad [\Delta G^0 = -30.5 \text{ KJ/mol}]$$

The ATP gets easily hydrolysed. This can be explained by the structures of both ATP and its hydrolysis products, ADP and Pi. ATP has very high phosphoryl transfer potential, i.e. the ability to transfer its phosphate group. At pH 7, the triphosphate unit of ATP carries about four negative charges. These charges repel each other strongly. This repulsion is reduced when ATP gets hydrolysed into more stable ADP and Pi. Hence, cells maintain a very high concentration of ATP so that its hydrolysis keeps releasing a great amount of energy. This energy is then used to carry various functions, which we have discussed in the beginning itself. On the other hand, ATP is formed by the transfer of phosphoryl group to ADP from other compounds. These compounds have a higher transfer potential than does ATP like phosphoenolpyruvate, acetyl released is then used to carry out various functions, which we have discussed in the beginning itself. Hence, ATP $\Leftrightarrow$ ADP cycle is the basis of energy exchange in all the organisms.
Chemistry of metabolism

A large number of cellular reactions involved in metabolism have been studied. It has been found that chemically they can be classified into six types of reactions only. These, along with their enzyme class are given in Table 1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Type of reaction</th>
<th>Description of reaction</th>
<th>Enzyme class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oxidation – reduction</td>
<td>Transfer of electrons</td>
<td>Oxidoreductases</td>
</tr>
<tr>
<td>2.</td>
<td>Group transfer</td>
<td>Transfer of a functional group from one molecule to another.</td>
<td>Transfases</td>
</tr>
<tr>
<td>3.</td>
<td>Hydrolysis</td>
<td>Cleavage of bonds by the addition of water.</td>
<td>Hydrolases</td>
</tr>
<tr>
<td>4.</td>
<td>Nonhydrolytic cleavage</td>
<td>Cleaving/splitting a molecule by nonhydrolytic processes.</td>
<td>Lyases</td>
</tr>
<tr>
<td>5.</td>
<td>Isomerization &amp; rearrangements</td>
<td>Rearrangement of atoms to form isomers.</td>
<td>Isomerases</td>
</tr>
<tr>
<td>6.</td>
<td>Ligation using energy from ATP</td>
<td>Formation of Carbon and other bonds with energy from ATP.</td>
<td>Ligases</td>
</tr>
</tbody>
</table>

(Source: Concepts in Biochemistry; Rodney Boyer)

Metabolic diversity in microbial world

We already know that:

(i) Green plants are photosynthetic. They obtain their energy from sunlight and use CO₂ as their carbon source.

(ii) Animals require preformed organic molecules for energy as well as building blocks.

We identify these plants and animal species on the basis of their size, shape and component structures. However, we cannot differentiate prokaryotic microorganisms from one another by their size or structure. They are generally differentiated from one another by their metabolism / nutrition. The nutrition includes processes by which the microbes take in and utilize food for energy and building material. The nutritional needs of microorganisms (mos) 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, CO₂ etc. Thus, the microbes can be divided into two groups based on their carbon sources:

(i) **Autotrophs** (Greek auto-self, trophe- nutrition): The organisms that derive carbon from CO₂.

(ii) **Heterotrophs** (Greek heteros – the other): The organisms using organic compounds as the carbon sources.

Similarly on the basis of energy sources, microbes are of two types:

(i) **Phototrophs** (Greek phos-light) are the organisms using light as energy source.

\[
ADP + Pi \xrightarrow{\text{Light}} ATP + H_2O
\]

(ii) **Chemotrophs** include the organisms carrying out chemical reactions to obtain the energy.
Besides carbon and energy sources, microorganisms also require a source of electrons for the reduction reactions.

(i) **Lithotrophs** (Greek *litho* – stone) - Microorganisms (mos) that derive the electrons from inorganic compounds like molecular hydrogen or reduced sulfur compounds.

(ii) **Organotrophs** – In these organisms, the electrons are donated by organic substrate.

Hence, nutritionally microbes can be divided into four major groups based on their source of carbon, energy and electron sources:

(i) Photolithotrophic autotrophs
(ii) Chemolithotrophic autotrophs
(iii) Photoorganotrophic heterotrophs
(iv) Chemoorganotrophic heterotrophs

Most of the bacterial species belong to only one of these four nutritional classes. However, some of them have the ability to change their metabolic patterns according to the changes in their surrounding environments, e.g.

(i) under normal oxygen levels, purple nonsulphur bacteria grow as chemotrophs. But, when oxygen is low, they act as photoorganotrophic heterotrophs.

(ii) most species of *Beggiatoa* use inorganic energy sources and (instead of CO₂) organic carbon sources because they lack enzymes of the Calvin cycle.

These microbes are often called as mixotrophs because they combine the chemolithoautotrophic and heterotrophic metabolic processes.

During metabolism, electrons are also released. Hence, electron acceptors are also required. Some mos use externally derived (exogenous) electron acceptors. This metabolic process is called respiration and may be divided into two different types. In aerobic respiration, the final electron acceptor is oxygen, whereas the acceptors in anaerobic respiration are usually nitrate (NO₃⁻), sulphate (SO₄²⁻), carbonate (CO₃²⁻), ferric iron (Fe³⁺) and even certain organic compounds. 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 (Latin *fermentare* – to cause, to rise or ferment).

In the present chapter we will discuss the metabolic pathways used by the different nutritional categories of microorganisms. This will include the pathways used by the microbes for:

(i) Generation of energy and reducing power, and

(ii) Assimilation of carbon source

**Chemoorganoheterotrophs**

These microorganisms undertake aerobic metabolism. The catabolism in these mos can be divided into 3 phases (Fig. 2).
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 CO$_2$, formation of NADH and FADH$_2$) followed by electron transport and oxidative phosphorylation. Energy released by electron transport of O$_2$ is coupled to ATP synthesis. This cycle is responsible for the release of much energy.

In this chapter, we will discuss various phases of breakdown of polysaccharides by mos. 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 mos 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.

**The Glycolytic Pathway**

The word glycolysis is derived from Greek words *Glyco* – sweet and *lysis* – splitting. 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 microorganisms. 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.

The detailed pathway showing the structures of the intermediate compounds, the enzymes involved in various reactions are shown in the Fig. 3. In the initial stages, phosphate group is added (phosphorylation) twice to the glucose and it gets converted to fructose 1,6-bisphosphate (F-1, 6-BP). Two ATPs are used in the process. The enzyme F-1,6-BP aldolase then splits the F-1, 6-BP into two three-carbon compounds: Glyceraldehyde – 3-phosphate (GAP) and Dihydroxyacetone phosphate (DHAP). These two compounds are interconvertible by the enzyme triosephosphate isomerase. The GAP gets converted directly to the pyruvate in a five-step process. During these steps some of the energy is extracted in the form of ATPs.

Fig. 3: Glycolysis/Embden – Meyerhof Pathway for the conversion of glucose and other sugars to pyruvate
(Source: Microbiology, 5th Edition; Prescott, Harley & Klein)
Now GAP is converted to 1,3 – bisphosphoglycerate (1, 3- BPG) by the addition of a phosphate group. In the next reaction, 1,3 – BPG donates one of its phosphate groups to ADP to produce ATP and itself gets converted to 3- phosphoglycerate (3-PG). The enzyme catalyzing this reaction is 3-PG kinase. The direct synthesis of ATP in such reactions is called substrate level phosphorylation because the phosphate donor i.e. 1,3-BPG is a substrate with high phosphoryl - transfer potential. A second ATP is also produced by the same process. The phosphate group in 3- phosphoglycerate gets rearranged resulting in the formation of 2- phosphoglycerate. In the next reaction, 2-phosphoglycerate gets dehydrated and the compound PEP is formed. Finally, PEP is converted to pyruvate by the enzyme pyruvate kinase. An ATP is also released during this reaction. The net reaction in the transformation of glucose into pyruvate is:

\[
\text{Glucose} + 2\text{P}_{i} + 2\text{ADP} + 2\text{NAD}^{+} \rightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^{+} + 2\text{H}_{2}\text{O}
\]

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

In this pathway shown in Fig. 4, 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. The key enzymes of ED pathway are:

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

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

**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, alongwith glycolysis or ED pathway. It can take place in aerobic or anaerobic conditions and is important in biosynthesis as well catabolism. The pathway (Fig. 5) involves 3 series of reactions:

(i) Oxidation of Glucose- 6- phosphate to ribulose -5-phosphate and formation of NADPH and CO$_2$.

\[
\text{Glucose} - 6 - \text{phosphate} + 2\text{NADP}^{+} \rightarrow \text{Ribulose} - 5 - \text{phosphate} + \text{CO}_2 + 2\text{NADPH} + 2\text{H}^{+}
\]

(ii) Reactions that allow formation of ribose- 5 -- phosphate and xylulose -5- phosphate from ribulose- 5- phosphate.

(iii) Reactions catalyzing the formation of hexoses from pentoses.
Fig. 4: The Entner-Doudoroff Pathway
(Source: Microbiology, 5th Edition; Prescott, Harley & Klein)
Fig. 5: The Pentose Phosphate Pathway
(Source: Microbiology, 5th Edition; Prescott, Harley & Klein)
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 \( \text{CO}_2 \). 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, one gly 3-P and 3 \( \text{CO}_2 \) molecules.

\[
3 \text{G-6-P} + 6\text{NADP}^+ + 3\text{H}_2\text{O} \rightarrow 2 \text{F-6-P} + \text{Gly-3-P} + 3\text{CO}_2 + 6\text{NADPH} + 6\text{H}^+ \\
\]

However, repetitive action of the cycle results in complete degradation of Glucose-6-phosphate to \( \text{CO}_2 \).

\[
\text{Glucose} - 6\text{-phosphate} + 12\text{NADP}^+ + 7\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 12\text{NADPH}^+ + 12\text{H}^+ + \text{P}_i \\
\]

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.

**Fate of pyruvate**

Pyruvate is a product of the three pathways of glucose catabolism –EMP, ED and Pentose Phosphate Pathways. In aerobic microorganisms, it undergoes further degradation and gets completely oxidized to \( \text{CO}_2 \) (phase III). However, to enter this phase, pyruvate undergoes some chemical changes brought about by a multienzyme complex called the Pyruvate dehydrogenase complex. This complex consists of 3 enzymes. These enzymes along with their coenzymes are as follows:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Coenzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>E₁ – Pyruvate dehydrogenase</td>
<td>Thiamine Pyrophosphate (TPP)</td>
</tr>
<tr>
<td>E₂ – Dihydrolipoate transacytase</td>
<td>Lipoamide &amp; Coenzyme A (CoASH)</td>
</tr>
<tr>
<td>E₃ – Dihydrolipoate dehydrogenase</td>
<td>Flavin Adenine Dinucleotide (FAD) &amp; Nicotinamide Adenine Dinucleotide (NAD)</td>
</tr>
</tbody>
</table>

The \( E_2 \) molecules are present in the center of the complex and \( E_1 \) and \( E_3 \) molecules are bound to the outside. This enzyme complex converts pyruvate into Acetyl-CoA by the following chemical changes:

(i) Decarboxylation of pyruvate (loss of \( \text{CO}_2 \))
(ii) Formation of acetyl group
(iii) Linkage of acetyl group to coenzyme A forming acetyl - CoA.
The detailed mechanism is shown in Fig. 6. The overall reaction is:

\[
\text{Pyruvate} + \text{CoASH} + \text{NAD}^+ \rightarrow \text{Acetyl CoA} + \text{NADH} + H^+ + \text{CO}_2.
\]

Acetyl CoA also arises from the catabolism of many carbohydrates, lipids and amino acids.

**Fig. 6: Reactions catalyzed by the pyruvate dehydrogenase complex.** $E_1$, pyruvate dehydrogenase; $E_2$, dihydrolipoate transacetylase; $E_3$, dihydrolipoate dehydrogenase; TPP, thiamine pyrophosphate; the disulfide compound linked to $E_2$ is the oxidized form of lipoate

(Source: Bacterial Metabolism; G. Gottschalk)

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:

1. **Bioenergetic** - The cycle carries out complex degradation of acetyl group in acetyl-CoA to CO$_2$, resulting in release of energy (ATP or GTP) and reducing power (NADH and FADH$_2$).
2. **Biosynthetic** - It supplies precursors for several biosynthetic pathways of amino acids, pyrimidines, purines etc.

The reactions involved in TCA cycle are given in Fig. 7. As can be seen, these reactions occur in cyclic manner. The cycle begins with the combination of acetyl – CoA (a two carbon, C$_2$, compound) and oxaloacetate (a four carbon, C$_4$, compound) to form citrate (a C$_6$ compd). The enzyme involved is citrate synthase. Citrate is rearranged to give isocitrate, which gets oxidized and decarboxylated to α-ketoglutarate (a C$_5$ compd). This C$_5$ compound now gets converted to succinyl - CoA (a C$_4$ compound). As is clear, during these steps two carbons are lost from the cycle as CO$_2$. There is formation of two NADHs. In the next step, the enzyme succinyl - CoA synthetase converts succinyl - CoA into succinate. Here, an GTP (or ATP) is formed by substrate – level phosphorylation. Subsequent steps lead to regeneration of oxaloacetate and formation of one FADH$_2$ and one NADH molecule. The important enzymes involved are fumarase and L-malate dehydrogenase. The net reaction of TCA cycle is:
Acetyl CoA + 3 NAD$^+$ + FAD + GDP + Pi + 2H$_2$O $\rightarrow$ 2CO$_2$ + 3NADH + 3H$^+$ + FADH$_2$ + GTP + CoA

The NADH and FADH$_2$ generated must be oxidized back to NAD and FAD, respectively. This will ensure the continuation of the TCA cycle. In aerobes, this is done by donating their hydrogens and electrons to oxygen (the H$^+$ and electron acceptor) via the respiratory chain. The process also leads to formation of ATP.

As already mentioned, the TCA cycle is also an important source of biosynthetic precursors e.g. α-ketoglutarate and oxaloacetate are used for synthesis of a number of amino acids like glutamic acid, asparatic acid etc. Succinyl - CoA is used to form porphyrin ring of cytochromes, chlorophyll etc. Oxaloacetate can also be converted to phosphoenolpyruvate, which is a precursor of glucose. Acetyl - CoA is the starting material for fatty acid
biosynthesis. These reactions remove intermediates from the citric acid cycle thereby effecting its efficiency. It has been found that microorganisms have some reactions that respell these intermediates to the TCA cycle. Such reactions that replace cycle intermediates are called as anaplerotic (“to fill in”) reactions. Usually these reactions involve CO₂ fixation. Various microorganisms use different enzymes for this purpose, e.g.

(i) *Arthrobacter globiformis* and yeasts:

\[
\text{Pyruvate carboxylase} \\
\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{Oxaloacetate} + \text{ADP} + \text{P}_i
\]

cofactor Biotin

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

\[
\text{PEP carboxylase} \\
\text{Phosphoenolpyruvate} + \text{CO}_2 \rightarrow \text{Oxaloacetate} + \text{P}_i
\]

**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 C₂ compd) and long chain fatty acids (which generate C₂ 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 anaplerotic reactions. In such cases, the oxaloacetate needed to continue the cycle is produced through the glyoxylate cycle (Fig. 8).

![Fig. 8: The Glyoxylate Cycle](Source: Lehninger Principles of Biochemistry; Nelson & Cox)
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.

**Respiratory / Electron Transport Chain**

Let's look back at the net reactions of glycolysis and TCA cycle. We observe that 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.

**Principle of ETC**

The electron carriers of the ETC transfer their electrons by undergoing oxidation – reduction process. The general reaction for this process is:

\[
\text{AH}_2 \rightleftharpoons \text{A} + n \text{electrons} \\
\text{(Reduced)} \quad \text{(Oxidized)}
\]

\[
\text{B} + \text{electrons} \rightleftharpoons \text{BH}_2 \\
\text{(Oxidized)} \quad \text{(Reduced)}
\]

As these are reversible reactions, their direction depends on the tendency of compound A to donate electrons to B and vice versa. This tendency is referred to as redox potential, $E^\circ$. $E^\circ$ of a compound is measured under standard temperature and pressure conditions and at a pH of 7. All compounds are compared to the redox potential of $\text{H}^+/\text{H}_2$ which is taken as 0.0V. Some compounds have positive $E^\circ$ values, i.e. they are better electron acceptors than $\text{H}^+$. On the other hand, some compounds have negative $E^\circ$ and they become better electron donors. The reduction potential of various electron carriers in ETC is given in the Table 2.

It is clear from the Table that the best reducing agent (electron donor) is NADH with $E^\circ = -0.32$V. The best oxidizing agent (electron acceptor) is oxygen with $E^\circ$ of +0.82 V.

In ETCs, the electron carriers are arranged in such a manner that electrons spontaneously flow from carriers with more negative $E^\circ$ to those having more positive redox potentials. The difference in reduction potentials between O$_2$ and NADH is about 1.14V. As a result, large amount of energy is released at various steps of electron transfer. This energy is calculated in terms of the free energy change, $\Delta G^\circ$

\[
\Delta G^\circ = -nFA E^\circ \text{ (KJ/mol), where}
\]

\[
n \quad = \quad \text{number of electrons transferred from one carrier to another.}
\]
F = the Faraday constant, 96.5 KJ/volt-mole.

ΔE₀' = the difference in E₀ of the electron acceptor and donor.

This released energy is used for the synthesis of ATP.

Table 2: Standard oxidation-reduction potentials of respiratory chain components at pH 7.0 and 30°C

<table>
<thead>
<tr>
<th>Component</th>
<th>E₀ (v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAD⁺ + 2H⁺ + 2e⁻ ⇌ NADH + H⁺</td>
<td>-0.320</td>
</tr>
<tr>
<td>FAD + 2H⁺ + 2e⁻ ⇌ FADH₂</td>
<td>-0.220</td>
</tr>
<tr>
<td>FMN + 2H⁺ + 2e⁻ ⇌ FMNH₂</td>
<td>-0.190</td>
</tr>
<tr>
<td>fumarate + 2H⁺ + 2e⁻ ⇌ succinate</td>
<td>+0.033</td>
</tr>
<tr>
<td>flavoproteins + 2H⁺ + 2e⁻ ⇌ red. flavoproteins</td>
<td>-0.450–0.0</td>
</tr>
<tr>
<td>FeS-proteins + 2e⁻ ⇌ red. FeS-proteins</td>
<td>-0.400–+0.200</td>
</tr>
<tr>
<td>menaquione + 2H⁺ + 2e⁻ ⇌ red. menaquione</td>
<td>-0.074</td>
</tr>
<tr>
<td>ubiquinone + 2H⁺ + 2e⁻ ⇌ red. ubiquinone</td>
<td>+0.113</td>
</tr>
<tr>
<td>2 cyt b ox + 2e⁻ ⇌ 2 cyt b red</td>
<td>+0.070</td>
</tr>
<tr>
<td>2 cyt c ox + 2e⁻ ⇌ 2 cyt c red</td>
<td>+0.254</td>
</tr>
<tr>
<td>2 cyt a ox + 2e⁻ ⇌ 2 cyt a red</td>
<td>+0.384</td>
</tr>
<tr>
<td>1/2O₂ + 2H⁺ + 2e⁻ ⇌ H₂O</td>
<td>+0.818</td>
</tr>
</tbody>
</table>

(Source: Microbial Physiology; Gerhard Gottschalk)

Components of ETC

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

1. **NADH dehydrogenase** accepts hydrogen atoms from NADH and pass to the flavoproteins.
2. **Flavoproteins** are proteins containing a derivative of vitamin – B riboflavin. Two flavoproteins are commonly involved in ETC – flavinmononucleotide (FMN) and flavin adenine dinucleotide (FAD). These accept hydrogen atoms and donate electrons.
3. **Iron-Sulphur (Fe-S) Proteins** are small molecules and contain equal amounts of iron and sulphur. Different Fe-S proteins have different redox potentials and function at different stages of electron transport e.g. Ferredoxin (Fd).
4. **Quinones** are nonprotein molecules, also written as coenzyme Q, CoQ or Q. Among these, Ubiquinones are present in ETC of eukaryotes and gram negative bacteria. Other bacteria (gram positive) contain menaquinone. Quinones are mobile electron carriers.
5. **Cytochromes** form a large group of proteins with iron-containing porphyrin ring called heme. These are characterized on the basis of their absorption spectra. They are designated as cyt a, cyt b, cyt c and so on.

The best example of a thoroughly studied ETC is the mitochondrial ETC. 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). The structure and function of these complexes is shown in Fig. 9a. Their arrangement according to their redox potential is shown in Fig. 9b.
Fig. 9a: Mitochondrial ETC
(Source: Concepts in Biochemistry; R. Boyer).

Fig. 9b: The Mitochondria Electron Transport Chain according to the redox potential
(Source: Microbiology, 5th Edition; Prescott, Harley & Klein)

The ETCs of mitochondria and that of microbial world share some properties. However, the major differences between the two are as follows:

1. Bacteria have a greater variety of electron carriers.
2. Many bacteria change the composition of their ETCs according to the changes in the growth conditions.
3. The bacterial respiratory chains can be branched especially in facultative anaerobes.

To understand the above points, we will consider the ETCs of *E. coli* and *Paracoccus denitrificans*. 
(a) **ETC of *E. coli* – *E. coli* is a gram-negative facultative anaerobe. When it is growing under aerobic conditions, the electrons flow via CoQ, cyt b562 and cyt 0. However, under limited oxygen conditions, it uses another series of electron carriers i.e. it uses CoQ, cyt b558 and cyt d (Fig. 10). But this chain produces less ATPs because it does not actively pump protons. It shows that ETC of *E. coli* is branched.

(b) **ETC of *P. denitrificans* – It is also a gram-negative facultative anaerobe. It is present in soil. *Paracoccus* carries out aerobic respiration, when growing on organic nutrients as heterotrophs. Its respiratory chain is typical for a bacterium growing aerobically. Also, it is similar to the respiratory chain in mitochondria (Fig. 11a).

![Fig. 10: The Aerobic Respiratory System of *E.coli*. The upper branch operates when the bacterium is in stationary phase and there is little oxygen. The lower branch functions when *E.coli* is growing rapidly with good aeration](source)

*P. denitrificans* can also grow as autotroph with H₂ and CO₂. Under these conditions, it carries out anaerobic respiration with nitrate as the electron acceptor. The composition of ETC, in these conditions, is very different. The cyt aa₃ complex does not function. Here, the electrons move from cyt c to nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Fig. 11b).

**Oxidative phosphorylation**

As already discussed, energy is released during the electron transfers from NADH (or FADH₂) to molecular oxygen. This energy is used to synthesize ATP by adding an inorganic phosphate to ADP (phosphorylation). This process is called as oxidative phosphorylation. Figure 12 shows the three coupling sites for oxidative phosphorylation at
the ETC of mitochondria. The number of ADPs phosphorylated per atom of oxygen used is expressed in terms of P/0 ratio. In mitochondrial chain, this ratio is 3 for NADH as electron donor. For FADH$_2$, it is two. In bacteria, the ratio varies with the composition of the ETC.

Fig. 11: *Paracoccus denitrificans* Electron Transport Chains. (a) The aerobic transport chain resembles a mitochondrial electron transport chain and uses oxygen as its acceptor. (b) The highly branched anaerobic chain. Nitrate is reduced to diatomic nitrogen by the collective action of four different reductases that receive electrons from CoQ and cytochrome c. Abbreviations used: flavoprotein (FP), methand dehydrogenase (MD), nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos) (Source: Microbiology, 5th Edition; Prescott, Harley & Klein)

The mechanism by which oxidative phosphorylation (or ATP synthesis) takes place is not very clearly known. In 1961, a British biochemist, Peter Mitchell proposed chemiosmotic hypothesis of ATP formation in an ETC. He received noble prize for the same in 1978.
It is a well-known fact that the electron carriers are located in the inner membrane of mitochondria. This hypothesis suggests that electron transport through the carriers causes the pumping of protons (H\(^+\) ions) from the inner mitochondrial matrix to the other side of the membrane. This pumping into the intermembrane space is always unidirectional. Hence, the concentration of H\(^+\) becomes higher in the intermembrane space than in the matrix. This leads to formation of a proton gradient. The proton gradient changes the charge on the inner membrane. That is the membrane becomes negatively charged because of movement of protons to the outside. This creates membrane potential. Together, the proton gradient and the membrane potential leads to formation of a proton motive force, PMF (ΔP), across the membrane. It is also called as an electrochemical potential. The PMF drives the synthesis of ATP by an enzyme complex, ATP synthase.

**ATP synthase**

ATP synthase is a large, complex membrane bound enzyme. It appears like knobs coming out of the inner membrane of mitochondria. The enzyme complex consists of two units F\(_1\) and F\(_0\). The F\(_1\) subunit is made of five polypeptide chains, \(α, β, γ, δ\) and \(ε\) (Fig. 13). It contains a catalytic site for ATP synthesis from ADP and Pi.

F\(_0\) subunit remains inserted in the mitochondrial membrane. It functions as a channel through which protons flow back. This movement drives the oxidative phosphorylation. In other words, ATP synthase couples the movement of protons with ATP synthesis. This is how an ATP is synthesized by the chemiosmotic mechanism.
Many chemicals can inhibit the ETC. They are of two types:-

Inhibitors are those chemicals which directly block the transport of electrons e.g. rotenone, amytal, HQNO (2-n-heptyl-4-hydroxy-quinoline-N-oxide), antimycin A, potassium cyanide, CO and azide. Site of action of these inhibitors is shown in Fig. 13a.

**Fig. 13a: Sites of action of electron transport inhibitors. Inhibitors and Uncouplers**

Uncouplers stop ATP synthesis without inhibiting electron transport. They make the membrane permeable for protons. As a result, PMF can’t be generated and ATP can’t be synthesized. Therefore, uncouplers are also called protonophores, e.g. Valinomycin and Dinitrophenol. However, arsenate (which is the pentavalent form of arsenic) is capable of uncoupling mitochondrial oxidative phosphorylation by a different mechanism. It is due to a competitive substitution of arsenate for inorganic phosphate and the formation of an arsenate ester, which is quickly hydrolysed.

**ATP yield during aerobic respiration**

When a molecule of glucose undergoes complete oxidation into CO₂ and H₂O under aerobic conditions, it passes through the glycolytic pathway and TCA cycle. The NAD (P)H and FADH₂ produced during this metabolism pass through nitrochondrial ETC and produce ATPs. Let us see how many ATPs are produced during this complete oxidation process.
**Glycolysis** – Breakdown of a glucose molecule into two pyruvates:

(i) Substrate level phosphorylation = 2 ATP  
(ii) Oxidative phosphorylation with 2 NADH = 6 ATP  

Conversion of 2 Pyruvate to 2 Acetyl – CoA  
Oxidative phosphorylation with 2 NADH = 6 ATP  

**TCA Cycle**

(i) Substrate level phosphorylation = 2 ATP  
(ii) Oxidative phosphorylation with 6 NADH = 18 ATP  
(iii) Oxidative phosphorylation with 2 FADH₂ = 4 ATP  

Total ATPs produced = 38 ATP  

As can be seen the net ATP produced with mitochondrial ETC are 38. However, the respiratory chains of mitochondria and microorganisms are very different. e.g. the *E.coli* ETC does not contain a cytochrome of the c-type and also, is branched. In addition, *E.coli* is a facultative anaerobe. So, even under aerobic conditions, a part of glucose undergoes fermentation. Hence, the net ATPs produced are actually less than 38. Similarly, ATP yield varies from microbe to microbe depending upon the components of their ETC.

**Chemolithotrophs**

Organisms that obtain energy from the oxidation of inorganic compounds are called chemolithotrophs. These bacteria are differentiated from one another on the basis of the inorganic compounds they oxidize e.g. nitrifying, sulphur, iron, hydrogen, carboxy-, methanogenic bacteria etc (Table 3). They can be both aerobic as well as anaerobic.

<table>
<thead>
<tr>
<th>Group</th>
<th>Energy Source</th>
<th>Product (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrifiers</td>
<td>NH₄⁺ / NO₂⁻</td>
<td>NO₃⁻ / NO₃⁻</td>
</tr>
<tr>
<td>Sulphur Oxidizers</td>
<td>H₂S, S⁰, S₂O₃²⁻</td>
<td>SO₄²⁻</td>
</tr>
<tr>
<td>Iron Oxidizers</td>
<td>Fe²⁺</td>
<td>Fe³⁺</td>
</tr>
<tr>
<td>Hydrogen Bacteria</td>
<td>H₂</td>
<td>H₂O</td>
</tr>
<tr>
<td>Carboxydobacteria</td>
<td>H₂ + CO</td>
<td>H₂O + CO₂</td>
</tr>
<tr>
<td>Methane Oxidizers</td>
<td>CH₄</td>
<td>CO₂ + H₂O</td>
</tr>
</tbody>
</table>

(Source: Microbial life; Perry, Staley & Lory)

Most of the chemolithotrophic bacteria use CO₂ as the sole source of carbon. However, some of them can also use organic compounds. Accordingly, they are of two types:

i) **Obligate Chemolithotrophs:**- They use CO₂ as the sole source of carbon. This carbon is used with the help of Calvin cycle (described later under the topic – Assimilation of CO₂.). These organisms are also called **chemolithoautotrophs.**

ii) **Facultative Chemolithotrophs:**- They fix CO₂ but can also use organic carbon sources. They are also called as **mixotrophs** (described with various nutritional
Energy production & generation of reducing power

For growth on CO$_2$ as the sole carbon source, an organism needs (1) energy (ATP), and (2) reducing power (NADH, NADPH). Most chemolithotrophs contain all the components of electron transport chain. Hence, they generate ATP in same manner as chemoorganoheterotrophs. The difference is that here the electron donor is inorganic (like H$_2$, NO$_2^-$, H$_2$S etc.) rather than an organic compound.

The oxidation of inorganic compounds by chemolithotrophs generates electrons and protons. The electrons pass through various membrane bound cytochromes resulting in generation of ATP by oxidative phosphorylation.

Even the protons formed are released outside the membrane generating proton motive force. This also results in the formation of ATP by a cell membrane ATPase.

The reducing power in some of the chemolithotrophs is obtained directly from the inorganic compound. The inorganic molecule, on its oxidation, donates electrons directly to NAD or NADP. However, in majority of the chemolithotrophs it is not possible as these compounds have more positive reduction potentials than NAD$^+$ / NADP$^+$. We already know that electrons spontaneously move only from donors with more negative potentials to acceptors with more positive potentials.

The chemolithotrophs solve this problem by a phenomenon known as reverse electron transfer. The mechanism of direct as well as reverse electron flow in *Nitrobacter* is shown in Figure 14. They use proton motive force to reverse the flow of electrons in their electron transport chains and reduce NAD$^+$ with electrons from the inorganic molecule. Because energy is used to generate NADH as well as ATP, the net yield of ATP is very low. That is why the chemolithotrophs are slow growers.

![Fig. 14: Electron Flow in Nitrobacter Electron Transport Chain](Source: Microbiology; Prescott, Harley & Klein)
**Nitrifying Bacteria:** They are the best-studied nitrogen-oxidizing chemolithotrophs. They use either ammonia (ammonia oxidizers) or nitrite (nitrite oxidizers) as their source of energy.

\[
\begin{align*}
\text{NH}_3 + 1\frac{1}{2}\text{O}_2 & \rightarrow \text{HNO}_2 + \text{H}_2\text{O} \\
\text{NO}_2^- + \text{H}_2\text{O} & \rightarrow \text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \\
\end{align*}
\]

The examples of ammonia oxidizers are *Nitrosomonas, Nitrosospira, Nitrosococcus* and nitrite oxidizers are *Nitrobacter, Nitrococcus, Nitrospira*.

The two genera work together resulting in oxidation of ammonia in the soil, first to nitrite and then to nitrate. The process is called as nitrification.

**Sulphur Oxidizers:** These bacteria can use sulphide, sulphur or thiosulphate as energy sources. Some can use all the three forms of sulphur. The sulphur oxidizers belong to two different groups – (i) the unicellular sulphur oxidizers viz. *Thiomicropira, Thermoithrix, Thiobacillus* (Fig. 15a) and (ii) the filamentous sulphur oxidizers, *Thioploca, Beggiatoa, Thioithrix* (Fig. 15b).

\[
\begin{align*}
\text{S}_2^- + 4\text{O}_2 & \rightarrow 2\text{SO}_4^{2-} \\
2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} & \rightarrow 2\text{H}_2\text{SO}_4 \\
\text{S}_2\text{O}_3^{2-} + 2\text{O}_2 + \text{H}_2\text{O} & \rightarrow 2\text{SO}_4^{2-} + 2\text{H}^+ \\
\end{align*}
\]

**Fig. 15a:** *Thiobacillus.* Thin section of rod-shaped cells of *Thiobacillus neapolitanus*

**Fig. 15 b:** *Thioithrix and Beggiatoa*
(Source: *Microbial life; Perry, Staley & Lory*)
The production of sulphate or sulphuric acid leads to the lowering of pH during the growth. Thus, they usually grow under acidic conditions.

The sulphur oxidizers are diverse metabolically. They can be obligate chemolithotrophs or mixotrophs. The examples include *Thiobacillus thiooxidans, T. ferrooxidans, T. denitrificans* (as chemolithoautotrophs) and *T. acidophilus, T. novellus, Sulfolobus acidocaldarius* (as mixotrophs).

**Iron Oxidizers:** Microorganisms that obtain energy by the oxidation of Ferrous iron (Fe$^{2+}$) are known as iron oxidizers. The oxidation takes place at acidic pH, optimum pH 2. Hence, these organisms are acidophilic.

\[
4 \text{FeSO}_4 + O_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2 \text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O}
\]

The examples are *Thiobacillus ferrooxidans, T. thiooxidans, Leptospirillum ferrooxidans* etc.

**Assimilation of CO$_2$ as carbon source**

Chemoautotrophs use CO$_2$ as their sole or principal carbon source. The reduction of CO$_2$ and its incorporation occurs via an anabolic pathway called as Calvin cycle.

The cycle was discovered by Calvin, Benson and Bassham in green algae. The other names for this cycle are Calvin-Benson cycle or reductive pentose phosphate cycle. It requires ATP and NAD(P)H. Their generation has already been discussed. The energy is obtained from oxidation of reduced inorganic electron donors. The NAD(P)H may be obtained directly from oxidation or by reverse electron transfer. The cycle (Fig. 16) can be divided into three phases: Carboxylation, Reduction and Regeneration.

(i) **The carboxylation phase** - During this phase, CO$_2$ is added to a 5- carbon sugar, ribulose 1,5 – bisphosphate (RuBP). The enzyme involved is ribulose 1,5-bisphosphate carboxylase (Rubisco). The product formed is two molecules of 3-phosphoglycerate (PGA).

(ii) **The reduction phase** - The PGA formed during the carboxylation phase, gets reduced to glyceraldehyde – 3 – phosphate. The reaction uses NADPH and is carried by two enzymes, phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase.

(iii) **The regeneration phase** - The first two phases result in conversion of one molecule of RuBP into 2 molecules of PGA. These PGA molecules are then used for biosynthesis. However, if this process continues as it is, it will lead to shortage of RuBP. Hence, the third phase is required which regenerates RuBP. This part of the cycle is almost similar to the pentose phosphate pathway and involves the transketolase and transaldolase reactions. One cycle requires 3 molecules of ATP and 2 molecules of NADPH. It is completed when phosphoribulokinase reforms RuBP.

To synthesize one molecule of fructose - 6 - phosphate from CO$_2$, the cycle operates six times i.e. it incorporates six molecules of CO$_2$ by following reaction:

\[
6 \text{RuBP} + 6\text{CO}_2 \rightarrow 6\text{RuBP} + 6 \text{Fructose - 6 - phosphate}
\]

The net reaction of Calvin cycle is:

\[
6 \text{CO}_2 + 18 \text{ATP} + 12 \text{NADPH} + 12\text{H}^+ + 12\text{H}_2\text{O} \rightarrow \text{glucose} + 18 \text{ADP} + 18\text{Pi} + 12 \text{NADP}^+
\]

Shively and coworkers discovered interesting organelles in *Thiobacillus neapolitanus*. Later, these were found in several obligate autotrophic prokaryotes that used Calvin cycle.
The organelles called carboxysomes are polyhedral inclusions, about 100 nm in diameter. They are clearly visible in *Thiobacillus* cells in Figure 15a. These are surrounded by a thin, nonunit membrane and consist mainly of Rubisco. It should be noted that Rubisco is the most abundant enzyme protein in nature.

Fig. 16: The Calvin Cycle
(Source: Microbiology, 5th Edition; Prescott, Harley & Klein)
**Phototrophs**

The organisms that obtain their chemical energy from light are called as Phototrophs. These include green plants, *cyanobacteria* and a few other phyla of bacteria. The process, which captures light energy and converts it to chemical energy is called photosynthesis. It provides photosynthetic organisms with the ATP and NADPH necessary for the anabolic metabolism.

The process of photosynthesis is divided into two parts:

**Light reactions:** The reactions in which light energy is trapped and converted to chemical energy.

**Dark reactions:** In these reactions the energy is used to reduce or fix CO$_2$ and synthesize cell constituents.

Some of the bacteria use CO$_2$ as their sole source of carbon and are called photolithotrophic autotrophs (*or photoautotrophs*). However, some of them require organic compounds as carbon sources and are called photoorganotrophic heterotrophs (*or photoheterotrophs*). Accordingly, they use different metabolic pathways for carbon assimilation. A list of photosynthetic bacteria alongwith their source(s) of carbon and pathways for carbon metabolism is given in Table 4.

<table>
<thead>
<tr>
<th>Phylum/Bacteria</th>
<th>Carbon Source</th>
<th>Carbon metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple sulphur</td>
<td>CO$_2$</td>
<td>Calvin-Benson cycle</td>
</tr>
<tr>
<td>Purple non sulphur</td>
<td>CO$_2$ and organics</td>
<td>Photoheterotrophic</td>
</tr>
<tr>
<td><strong>Chlorobi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green sulphur</td>
<td>CO$_2$</td>
<td>Reductive TCA cycle</td>
</tr>
<tr>
<td><strong>Chloroflexi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green filamentous</td>
<td>CO$_2$ and organics</td>
<td>Hydroxypropionate pathway</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td>CO$_2$</td>
<td>Calvin-Benson cycle</td>
</tr>
<tr>
<td><strong>Firmicutes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Helioibacteria</strong></td>
<td>Organics</td>
<td>Photoheterotrophic</td>
</tr>
</tbody>
</table>

(Source: Microbial life; Perry, Staley & Lory)

**Light reactions**

**Light reactions in Cyanobacteria**

The *cyanobacteria* (previously also called blue – green algae) comprise a large group of organisms. They are typical prokaryotes with 70S ribosomes and with peptidoglycan as cell wall constituent. Examples of unicellular cyanobacteria are *Synechococcus*, *Gloeocapsa* species and filamentous forms are *Oscillatoria*, *Anabaena*, *Spirulina* spp.

The process of photosynthesis in *Cyanobacteria* is similar to that of higher plants. Like plants, *Cyanobacteria* use light as energy source, water as electron donor and CO$_2$ as carbon source. Since the process also leads to evolution of oxygen, it is called as oxygenic photosynthesis. That the oxygen is evolved during photosynthesis was discovered by Robert Hill during 1930s.
The photosynthetic apparatus in *Cyanobacteria* is located in intracellular / lamellar membranes called thylakoids. These membranes are studded with small structures called phycobilisomes, which contain phycobiliproteins. The apparatus is comprised of two systems, which absorb light at different wavelengths. They are Photosystem (PS) I (≥ 680 nm) and PS II (≤ 680 nm). These photosystems were discovered by Robert Emerson in 1940s. As a result, photosynthesis in cyanobacteria comprises two light reactions.

Light is absorbed by the antenna or light harvesting (LH) centers present in the two photosystems. They consist of a large number of chlorophyll *a* molecules, and accessory pigments like carotenoids and phycobiliproteins. From these LH centers the energy is transferred to special chlorophyll present in the reaction center. In case of PS I, it is called as P700 and in PS II as P680. These molecules get excited upon absorbing the energy.

The excited electron from P 700 gets transported to various electron carriers in a cyclic manner. These include an iron – sulphur protein, ferredoxin (Fd), plastoquinone (PQ), an iron-sulphur – cytochrome *bf* complex and plastocyanin (PC). Finally, the electron returns back to P 700 (Fig. 17). PMF is formed during this cyclic electron transport in the region of cyt *bf* complex and used to synthesize ATP. This process is called cyclic photophosphorylation.

---

**Fig. 17:** Electron flow in oxygenic (green plant) photosynthesis, the “Z” scheme. Two photosystems (PS) are involved, PS I and PS II. Ph, Pheophytin; Q, quinone; Chl, chlorophyll *a*; Cyt, cytochrome; PC, plastocyanin; FeS, nonheme iron-sulfur protein; Fd, ferredoxin; Fp, flavo-protein; P680 and P700 are the reaction center chlorophylls of PS II and PS I, respectively

(Source: Biology of microorganisms; Brock & Co.)
During the cyclic electron transfer, the electrons are also taken up by NAD(P) for generating NAD(P)H. This disrupts the cyclic pathway. Thus, to continue this pathway, an external electron donor is required. At this stage PS II donates electrons to the oxidized P700, generating ATP in the process. This is called noncyclic photophosphorylation. The LH center of PS II absorbs light and excites P680. The excited electron leaves P680 and travels to pheophytin and then to quinone and PC of PS I (Fig. 17).

One ATP and one NADPH are formed when two electrons travel through the noncyclic pathway. The oxidized P680 gets an electron from the “splitting of water” causing release of O\textsubscript{2}.

As is clear, the photosynthetic electron transfer chain is a series of electron carriers like ETC in aerobic organisms. Also, the electrons are transferred from one carrier to another, thereby, increasing their redox potential. These carriers of photosynthetic ETC if arranged according to their redox potential show a zigzag or Z-shaped pattern. This pattern is also referred to as Z-scheme (Fig. 17).

**Light reactions in phototrophic bacteria**

The phototrophic bacteria fall into four major groups: (i) the photosynthetic Proteobacteria, (ii) Chlorobi (green sulphur bacteria), (iii) Chloroflexi (green filamentous bacteria) and (iv) the photosynthetic Firmicutes (heliobacteria).

The mechanism of photosynthesis in these bacterial groups is different from that in Cyanobacteria in following ways:

(i) It does not result in release of oxygen. Therefore, it is referred to as anoxygenic photosynthesis.

\[
\begin{align*}
\text{H}_2\text{S} + \text{CO}_2 & \rightarrow (\text{CH}_2\text{O})_n + \text{S}^0 \\
\text{S}^0 + \text{CO}_2 + \text{H}_2\text{O} & \rightarrow (\text{CH}_2\text{O})_n + \text{H}_2\text{SO}_4
\end{align*}
\]

(ii) The process occurs strictly under anaerobic conditions.

(iii) It is noncyclic.

(iv) It involves only one photosystem that is PS I.

(v) PS I is comprised of bacteriochlorophyll (Bchl) molecules instead of chlorophylls. Five Bchls, viz a,b,c,d, and e are known in different phototrophic bacteria.

(vi) They use electron donors like hydrogen, hydrogen sulphide, elemental sulphur and organic compounds.

**The photosynthetic Proteobacteria:** They are commonly called purple bacteria because of purplish colour of carotenoids present in them. This colour masks the green colour of Bchl a/b. These bacteria comprise two separate subgroups: the purple sulphur bacteria and the purple nonsulphur bacteria. The sulphur bacteria utilize H\textsubscript{2}S in photosynthesis and form elemental sulphur granules inside their cells e.g. *Chromatium, Thiospirillum, Thiodictyon, Thiopedia* etc.

The purple nonsulphur bacteria can grow aerobically as chemoheterotrophs using certain organic substrates. But under anaerobic conditions in light, they carry out photosynthesis e.g. *Rhodobacter, Rhodopseudomonas, Rhodospirillum*. The process of photosynthesis is same in both the subgroups. The photosynthetic apparatus in both the subgroups consists of
a series of intracytoplasmic membranes (ICMs). These ICMs are vesicular extensions of the cytoplasmic membrane. The LH centers of purple bacteria (present on ICMs) consist of Bchls, carotenoids and several proteins. Two types of LH centers are present – B850 and B875. They absorb light and transfer it to a “special pair” of Bchl in reaction centers (RCs). The RC in purple bacteria is designated as P 870. As a result the special pair gets excited and becomes a strong electron donor. RC has four Bchl $a$ molecules, 2 molecules of bacteriopheophytin (Bchl $a$ minus Mg atom), 2 molecules of quinone and 2 molecules of a carotenoid pigment. Within the RC, electron moves to bacteriopheophytin and quinone molecules. Then the electrons are transported through a series of iron- sulphur proteins and cytochromes. The important cytochromes include cyt. $bc_1$, and cyt. $c_2$ Finally, it returns to the RC (Fig. 18).

![Fig. 18: A comparison of electron flow in purple bacteria, green sulfur bacteria, and heliobacteria](Source: Biology of micro-organisms, Brock & Co.)

During this cyclic electron transport, protons get translocated from inside to outside the membrane. As a result, PMF is formed and ATP synthesis occurs. The bacteria also require reducing power (NADH or NADPH) so that CO$_2$ can be used to make cell material. For this purpose, H$_2$S, S$^0$ and organic compounds are used as H-donors. These substances are oxidized by cytochromes and via quinones reduce NAD$^+$ to NADH. Since $E'_0$ of quinone is not negative enough (-0.32 V), energy is utilized for this reversed electron flow.

In this manner, purple bacteria obtain ATP by cyclic photophosphorylation and NADH by reverse electron flow.

**Chlorobi and Chloroflexi (Green Bacteria):** Green bacteria are strict anaerobic organisms that are obligately photosynthetic. Their green colour is due to presence of Bchls ($c$, $d$ or $e$). They utilize H$_2$S, thiosulphate or H$_2$ as an electron donor.

*Chlorobi* are the green sulphur bacteria e.g. *Chlorobium, Pelodictyon, Prostheocochloris* while *Chloroflexi* are filamentous and multicellular organisms like *Chloroflexus, Heliothrix, Oscillochloris.*
The photosynthetic apparatus in green bacteria is present in special organelles, called as chlorosomes. These are vesicles surrounded by a non-unit membrane and lie beneath the cytoplasmic membrane. The LH centers absorb light and transfer it to the RCs (P840) that are situated in the cytoplasmic membrane. The cyclic electron flow resulting in ATP synthesis is shown in Fig. 18. Here, ferredoxin (Fd) serves directly as electron donor and reduces NAD to NADH. Because of this, an electron hole is created. This hole is filled by an external electron donor. This is accomplished by sulfide oxidation and simultaneous reduction of cytochromes.

**The Phototrophic Firmicutes:** The heliobacteria, members of Firmicutes are the most recently discovered photosynthetic bacteria. They are the first group of gram – positive phototrophic prokaryotes. The cells are red-brown in colour due to the carotenoid pigment, neurosporene e.g. *Heliobacterium* and *Heliobacillus*.

The heliobacteria are all photoheterotrophic i.e. they require an organic carbon source for growth. Their reaction centers (P798) contain Bchl g (a modified Chl. a). Figure 18 shows the cyclic electron flow in heliobacteria. The formation of reducing power, like green bacteria, is directly by Fd.

**Carbon metabolism in phototrophs**

Like chemoautotrophs, phototrophs also use CO$_2$ as their sole or principal carbon source. However, they fix and assimilate CO$_2$ by either of the three ways:
(i) Calvin cycle
(ii) Reductive TCA cycle
(iii) Hydroxypropionate pathway

The incorporation of CO$_2$ requires much energy, which is obtained by trapping light during photosynthesis. Autotrophic CO$_2$ fixation is very important to life on earth because it provides the organic matter for heterotrophs.

**Calvin Cycle:** The cycle forms the dark reaction of photosynthesis. It is found in photosynthetic eukaryotes and most photosynthetic prokaryotes like *Cyanobacteria*, *Proteobacteria*, Chemoautotrophs, etc. The mechanism of the cycle has already been discussed in chemotrophs.

**Reductive TCA cycle:** The green sulphur bacteria, *Chlorobi* fix CO$_2$ by the reductive TCA cycle. The cycle was first given by Arnon, Buchanan and Evans. It is almost reversal of the TCA cycle. It utilizes ATP and NADH to form acetyl-CoA from CO$_2$.

Since most of the reactions of TCA cycle are reversible, the reductive TCA cycle is almost its reversal (Fig. 19a). However, in three of the steps, the enzymes are replaced:
(i) Succinate dehydrogenase is replaced by fumarate reductase.
(ii) $\alpha$ - Ketoglutarate dehydrogenase by $\alpha$ - ketoglutarate synthase.
(iii) Citrate synthase by ATP – citrate lyase.

The result of the cycle is that 2 CO$_2$ are reduced to acetyl - CoA. It is then carboxylated to pyruvate, which then enters various biosynthetic pathways.

**The hydroxypropionate pathway:** The green nonsulphur phototrophs, *Chloroflexi*, use the hydroxypropionate pathway for CO$_2$ reduction and assimilation. Hydroxypropionyl – CoA
and propionyl – CoA are the key intermediates (Fig. 19b). One cycle leads to formation of glyoxylate, which then enters various biosynthetic pathways.

Fig. 19: Unique autotrophic pathways in phototrophic green bacteria: (a) The reverse citric acid cycle is the mechanism of CO₂ fixation in the green sulfur bacterium Chlorobium; (b) The hydroxypropionate pathway is the means of autotrophy in the green nonsulfur bacterium Chloroflexus
(Source: Biology of microorganisms; Brock & Co.)

Photosynthesis without chlorophyll

Photosynthesis without chlorophyll occurs in a group of archaean bacteria known as Halobacteria. These bacteria are unique as they live in highly saline environments like salt lakes. They require concentration of >2.5 M NaCl to grow.
Halobacteria are obligate aerobes and have a membrane-bound ETC for ATP generation. Their membrane is red and contains carotenoids. When growing below the surface of water, the halobacteria encounter conditions of low oxygen concentration. This is because of low solubility of oxygen in concentrated solutions. Under such conditions, they synthesize a large number of purple patches within their cytoplasmic membrane. Stoeckensius and coworkers found that these patches consist of bacteriorhodopsin. It is a protein along with a coloured compound, retinal. The bacteriorhodopsin absorbs light at wavelength 570 nm and throws protons outward. In the process, it gets bleached. The proton is taken back in a dark reaction. Thus, an electrochemical gradient is established across the membrane and is used for ATP synthesis (Fig. 20). In brief, the photosynthesis in halobacteria is a simple light driven proton pump.

![Fig. 20: Light-driven proton pump of halophilic bacteria](Source: Microbial life; Perry, Staley & Lory)

**Fermentation**

We have already studied that one molecule of glucose is oxidized first to two molecules of pyruvate by glycolysis. The TCA cycle and ETC, then, completely metabolize this pyruvate. This occurs either in the presence of oxygen (aerobic respiration) or other electron acceptors like NO$_3^-$, SO$_4^{2-}$, CO$_3^{2-}$, etc. (anerobic respiration). ETC also regenerates NAD by oxidizing NADH produced during these pathways. This ensures that the metabolic processes continue.

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. Basically, the process of fermentation includes:

- Conversion of NADH to NAD
- Generation of ATP
- An electron acceptor, which is usually pyruvate or its derivative.

The terms “fermentation” was first defined by Pasteur as life without oxygen. Following are the features of fermentation:

- It is an energy yielding process.
- It is carried out by either facultative or obligate anaerobes.
- There is absence of ETC in fermentation. Therefore, O$_2$ is not required.
- During fermentation, one organic compound (i.e. the substrate) serves as a donor of electrons and another organic compound (the product) is the electron acceptor.
- The electron donor and acceptor are neutral in the redox state i.e. they are neither highly oxidized nor highly reduced.
- The organic substrate in fermentation gets only partially oxidized. Therefore, only a limited amount of ATP is formed.
- ATP synthesis occurs by substrate level phosphorylation.

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.

**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:

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

(Sugar) (Ethanol)

Figure 21 summarizes the ethanol fermentation pathway. 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, as shown in the Fig. 21.

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. Most of them have complex nutritional requirements.
Fig. 21: Fermentation of glucose to ethanol and CO$_2$ by yeasts: 1- Initial enzymes of the Embden-Meyerhof-Parnas pathway; 2- glyceraldehyde-3-phosphate dehydrogenase; 3- 3-phosphoglycerate kinase; 4- phosphoglycerate mutase; 5- enolase; 6- pyruvate kinase; 7- pyruvate decarboxylase; 8- alcohol dehydrogenase
(Source: Bacterial Metabolism; G. Gottschalk)

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

1. The **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 $\rightarrow$ 2 Lactic acid.

As can be seen in Fig. 22a, the EMP pathway is used in this process. The pyruvic acid formed is reduced by the enzyme lactate dehydrogenase to lactic acid. Here also 2 ATPs are generated for one glucose molecule.

2. The **heterolactic / heterofermentative bacteria** e.g. *Lactobacillus brevis*, *L. fermentum*, *Leuconostoc*. These bacteria produce ethanol and CO$_2$ along with the lactic acid.

Glucose $\rightarrow$ Lactic acid + Ethanol + CO$_2$

The heterofermentative bacteria do not use glycolysis, but use a phosphoketolase pathway for fermentation. The detailed pathway is shown in Fig. 22b. Only one ATP is produced per molecule of glucose.

In addition to these two fermentations, another bacterial species *Bifidobacterium bifidum* produces lactic acid by following another pathway. The pathway is called as bifidum pathway (Fig. 22c). It produces a mixture of acetic acid and lactic acid by the equation:

2 glucose $\rightarrow$ 3 Acetic acid + 2 Lactic acid

Both alcoholic and lactic acid fermentations are quite useful. The alcoholic fermentation produces alcoholic beverages. The CO$_2$ produced causes bread to rise. Lactic acid...
fermentation can spoil foods. But it is also used for making yogurt, pickles etc. Besides these sugars bacteria can also ferment amino acids and organic acids like acetic acid, lactic acid, citric acid etc.

Fig. 22 a: Formation of lactate from glucose by the homofermentative pathway. 1- Enzymes of the Embden-Meyerhof-Parnas pathway; 2- lactate dehydrogenase

Fig. 22b: Formation of CO$_2$, lactate, and ethanol from glucose by the heterofermentative pathway, 1- Hexokinase; 2- Glucose-6-phosphate dehydrogenase; 3- 6-phosphogluconate dehydrogenase; 4- ribulose-5-phosphate 3-epimerase; 5- phosphoketolase. The cleavage reaction yields glyceraldehyde-3-phosphate and enzyme-bound $\alpha$, $\beta$-dihydroxyethyl-thiamine pyrophosphate. This is converted to acetyl-TPP-E via the $\alpha$-hydroxyvinyl derivative; phosphorylytic cleavage results in acetyl phosphate formation. 6- phosphotransa cetylase; 7- acetaldehyde dehydrogenase; 8- alcohol dehydrogenase; 9- enzymes as in homofermentative pathway (Source: Bacterial Metabolism; G. Gottschalk)
Methane fermentation

Methane is the most reduced organic compound. It is produced during the last step of the anaerobic food chain by a process called as methanogenesis or methane fermentation. Hence, methanogenesis is a significant component of carbon cycle on earth. This process is carried out by some archael bacteria, referred to as methanogens. They convert CO₂, H₂, formate, methanol, acetate etc. to methane or methane and CO₂. However, these bacteria can’t utilize complex organic compounds. Some of the important features of methanogens are:

1) They belong to Archea.
2) They are obligate anaerobic chemolithoautotrophs.
3) Their metabolism is unusual.

Methanogens are extremely oxygen-sensitive. They live in anaerobic environments which are rich in organic matter e.g. the swamps, digesters, the rumen and the intestinal system of animals, freshwater and marine sediments etc. On the basis of the compounds they reduce, methanogens can be classified into 2 groups:

1. **Obligate methanoanogens** – They utilize $\text{CO}_2$ and $\text{H}_2$ to produce methane.

$$\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}.$$ 

Some of these also grow with formate and CO, e.g. *Methanobacterium thermoautotrophicum*, *Methanobrevibacter arboriphilus*, *Methanococcus vanniellii*

2. **Methylotrophic methanogens** – These grow with methyl - group containing substrates viz. methanol, methylamines, acetate etc.

$$4 \text{CH}_3 \text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 2\text{H}_2\text{O}$$

(methanol)    (methane)

$$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$$

(acetate)    (methane)

*e.g. Methanosarcina barkeri*, *Methanosarcina mazei*, *Methanothrix soehngenii*.

It has been found that group (2) organisms produce methane directly from the methyl groups and not via $\text{CO}_2$.

**Novel Coenzymes of Methanogens**

Methanogens have been found to have several unusual coenzymes. They are:

(i) Methanofuran (MFR) – It acts as primary $\text{CO}_2$ acceptor. It contains a furan ring and an amino nitrogen atom that binds $\text{CO}_2$.

(ii) Tetrahydromethanopterin ($\text{H}_4\text{MPT}$) – It resembles folic acid.

(iii) Coenzyme $\text{F}_{420}$ – It carries electrons and hydrogens.

(iv) Coenzyme $\text{F}_{430}$ - It is a nickel – containing tetrapyrrrole. This coenzyme acts as cofactor for the enzyme methyl – $\text{CoM}$ methyl reductase.

(v) Coenzyme $\text{M}$ (CoM) – It is a simple chemical compound i.e. 2-mercaptoethane sulfonic acid. Its reactive group is mercapto group.

(vi) 7- mercaptothreonine phosphate (or Coenzyme B, CoB)- It is a thiol containing coenzyme. It contains a threonyl phosphate residue.

The structure of these coenzymes is given in Fig. 23.

**Methane formation from $\text{CO}_2$**

The steps in production of methane from $\text{CO}_2$ are shown in Figure-24a and are summarized as follows:

(i) Initially $\text{CO}_2$ binds methanofuran and is reduced to formyl group.

(ii) The formyl group is then transferred to tetrahyromethanopterin and gets reduced to methyl group.

(iii) The methyl group is then transferred to Coenzyme M forming methyl- $\text{CoM}$.

(iv) In the last reaction, methyl- $\text{CoM}$ is reduced to methane by methyl reductase. This enzyme requires two additional coenzymes, $\text{F}_{430}$ and CoB.
As we can see from the pathway (Fig. 24a), three coupling sites exist for the generation of a PMF at the membrane. It is used by ATP synthase to produce ATP.

**Methanogenesis from other substrates**

The pathway of methane synthesis from methyl compounds and acetate is different from methane formation from CO\textsubscript{2}. However, methyl – CoM is an important intermediate in this pathway as well.

(i) For methyl compounds, the methyl group is first transferred to a cobamide-containing (or corrinoid) protein and then to CoM. Three fourths of methyl groups are then reduced to methane and one fourth oxidized to CO\textsubscript{2}.

(ii) When acetate is the substrate for methanogenesis, it is first activated to acetyl Co-A. The acetyl- CoA is then cleaved by an enzyme complex, CO dehydrogenase. The cleavage results in formation of an enzyme-bound methyl group, an enzyme bound CO and Coenzyme A. The methyl group is transferred to CoM by tetrahydromethanopterin (CoB). The enzyme-bound CO is oxidized to CO\textsubscript{2}. Here Fd acts as the electron carrier and gets reduced. At this step an ATP is also formed. The Fd is then used to reduce CH\textsubscript{3}-S-CoM to methane (Fig. 24b).

In nature, various complex substrates like cellulose, starch or protein are degraded to acetate, propionate, butyrate, alcohol, H\textsubscript{2}, CO\textsubscript{2} etc. by a number of anaerobic bacteria. Methanogens grow in the vicinity of these fermenters and use H\textsubscript{2} produced by them for
methane formation. This interaction between H₂-producers and H₂-consumers is referred to as interspecies hydrogen transfer. This is a good example of syntrophy, an ecological interaction.

Fig. 24: Pathways of methanogenesis: (A) Methyl-coenzyme M is a central intermediate in methanogenesis from CO₂, acetate, and methanol or methylamine; (B) The aceticlastic reaction for biosynthesis of methyltetra-hydromethanopterin from acetate (Source: Microbial life; Perry, Staley & Lory)
Nitrogen metabolism

All living organisms require nitrogen because it is an important constituent of proteins, nucleic acids etc. Animals use only organic nitrogen sources. Plants use only inorganic nitrogen sources such as ammonia or nitrate. Microorganisms use both organic as well as inorganic nitrogen sources. They also have the ability to degrade various nitrogenous compounds. This degradation causes return of nitrogen to the nitrogen cycle. At the same time, some bacteria have unique property of fixing atmospheric nitrogen into ammonia. The so formed ammonia gets added into various compounds, which are then utilized by eukaryotes. This way microorganisms play several important roles in the nitrogen cycle. They are responsible for several processes not carried out by other organisms.

Nitrogen fixation

The conversion of atmospheric nitrogen gas to ammonia is called nitrogen fixation. It is a reduction process. Only a few prokaryotic organisms can carry out nitrogen fixation. They include (i) free-living bacteria (Azotobacter, Klebsiella, Clostridium, Methanococcus), (ii) bacteria living in symbiotic association with plants (Rhizobium, Bradyrhizobium) and (iii) Cyanobacteria (Nostoc, Anabaena). A detailed list of these organisms is given in Table 5. Azotobacter and Rhizobium were isolated by Matinus W. Beijerinck (1851-1931).

Table 5: Examples of Nitrogen-Fixing Organisms

<table>
<thead>
<tr>
<th>Symbiotic Association of Various Genera with Leguminous Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azorhizobium caulindans, tropical legume (Sesbania rostrata)</td>
</tr>
<tr>
<td>Bradyrhizobium japonicum, soyabean (Glycine max)</td>
</tr>
<tr>
<td>Rhizobium trifolii, clover (Trifolium crotalaria)</td>
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</tbody>
</table>

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<tr>
<th>Symbiotic Association of Actinomycetes with Angiosperms</th>
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</thead>
<tbody>
<tr>
<td>Frankia sp., alder (Alnus)</td>
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<tr>
<td>Frankia sp., bog myrtle or sweet gale (Myrica)</td>
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</tbody>
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<tr>
<th>Symbiotic Association with Leaf-Nodulating Plants</th>
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<tbody>
<tr>
<td>Klebsiella aerogenes</td>
</tr>
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<tr>
<th>Associative Interaction with Grasses</th>
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</thead>
<tbody>
<tr>
<td>Azospirillum brasiliense, tropical grasses</td>
</tr>
<tr>
<td>Azospirillum lipoferum, tropical grasses, maize</td>
</tr>
<tr>
<td>Azotobacter paspali, tropical grass (Paspalum notatum)</td>
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</tbody>
</table>

<table>
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<tr>
<th>Free-Living Bacteria and Cyanobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic, heterotrophic</td>
</tr>
<tr>
<td>Azotobacter, Biejerinkia, Nocardia, Pseudomonas</td>
</tr>
<tr>
<td>Aerobic, Phototrophic</td>
</tr>
<tr>
<td>Anabaena, Calothrix, Nostoc, Gleotheca</td>
</tr>
<tr>
<td>Facultative, heterotrophic</td>
</tr>
<tr>
<td>Enterobacter cloacae, Klebsiella pneumoniae, Bacillus polymyx,</td>
</tr>
<tr>
<td>Anaerobic, heterotrophic</td>
</tr>
<tr>
<td>Clostridium butyricum</td>
</tr>
</tbody>
</table>

| Anaerobic, phototrophic                                      |
Chromatium vinosum, Rhodospirillum rubrum, Rhodopseudomonas sphaeroides, R. capsulate, Rhodomicrobium vernielli, Rhodocyclus, Chlorobium limocola

Nonphotosynthetic, autotrophic

Methanobacterium, Methylococcus, Methanococcus, Methanosarcina

(Source: Microbial Physiology; Moat, Foster & Spector)

The process of nitrogen fixation in these organisms is catalyzed by the enzyme nitrogenase. Nitrogenase is a multicomponent enzyme system. It consists of two proteins called component I (or dinitrogenase) and component II (or dinitrogenase reductase). Component I has molecular weight of 220,000D and contains iron and two atoms of molybdenum. Component II has molecular weight of 64,000D and contains four iron atoms. Therefore, the two components are also called as MoFe protein and Fe protein, respectively. The formation of ammonia from \( \text{N}_2 \) and \( \text{H}_2 \) is as follows:

\[
\text{N}_2 + 3 \text{H}_2 \rightarrow 2 \text{NH}_3
\]

This reaction is an exothermic reaction i.e. it releases energy (\( \Delta H = -91.2 \text{ KJ} \)). However, the reaction also requires lots of energy in the form of ATP. This is because the nitrogen gas has triple bonds between two nitrogen atoms (\( \text{N} \equiv \text{N} \)). These bonds are very difficult to break and hence, require very high dissociation energy. The net reaction of \( \text{N}_2 \) fixation is:

\[
\text{N}_2 + 8 \text{H}^+ + 8\text{e}^- + 16 \text{ATP} \rightarrow 2 \text{NH}_3 + \text{H}_2 + 16 \text{ADP} + 16 \text{Pi}
\]

The hydrogen gas has been found to evolve during the nitrogenase reaction.

The reaction requires reduced ferredoxin or flavodoxin as electron source. These reduced ferredoxin or flavodoxin molecules transfer electrons to Fe protein (component II), thereby, reducing it. ATP binds to this reduced molecule causing decrease in its redox potential. Now the reduced Fe protein can transfer its electrons to MoFe protein (component II). ATP gets hydrolyzed after this electron transfer. The reduced MoFe protein donates its electrons to \( \text{N}_2 \) and converts it to \( \text{NH}_3 \). Hence, nitrogen fixation is highly reductive in nature. Its mechanism is diagrammatically represented in Fig. 25.

Nitrogenases from various microbes have been found to be highly sensitive to \( \text{O}_2 \). The enzyme gets inactivated by \( \text{O}_2 \). Hence, it is a problem for all aerobic nitrogen fixers to keep the nitrogenase system anaerobic. To overcome this problem, they have evolved various mechanisms to keep \( \text{O}_2 \) away from this enzyme system. Some of these include:

(i) Rapid removal of \( \text{O}_2 \) by increasing rate of respiration.
(ii) Production of slime layers through which \( \text{O}_2 \) can’t pass easily.
(iii) The presence of heterocysts (specialized anaerobic cells) in Cyanobacteria etc.

**Symbiotic nitrogen fixation**

Some bacteria infect roots of the leguminous plants and form colonies. This infection results in the formation of root nodules. The bacteria in these nodules fix nitrogen for use by the plant. The plant, in turn, provides them with organic substrates like malate, succinate, fumarate etc. This type of symbiotic association is shown by two major genera of bacteria, Rhizobium and Bradyrhizobium.
The pictures of leguminous plants showing root nodules were drawn by Marcello Malpighi (1628-1694), an Italian anatomist. In 1888, Martinus Beijerinck demonstrated that bacteria formed root nodules. The formation of root nodules in *Rhizobium* – plant symbiosis occurs as follows (Fig. 26):

i) The plant root releases chemicals called flavonoids. These attract *Rhizobium* and induce their *nod* (nodule) genes.

ii) The factors released by the *nod* genes of bacteria stimulate cell division in the root.

iii) At the same time, the bacteria get attached to root hairs. This causes the root hairs to curl.

iv) The attached bacteria induce the formation of an infection thread that grows down the root hair. Through this thread, the bacteria move, divide and infect the cells around the infection thread.

v) Within the root cells, most of the bacteria get irregular shaped and are called bacteroids. The bacteroids are incapable of cell division.

vi) The bacteroids also get covered by a membrane which is derived from plants. Now, they are known as symbiosomes. Only symbiosomes can fix nitrogen.

vii) At this stage, a red pigment called leghemoglobin is produced. This is an iron-containing heme protein. Its function is to protect nitrogenase from oxygen.

The production of leghemoglobin completes the formation of root nodules.
Regulation of nitrogenase

The activity of nitrogenase is inhibited by (i) high levels of ammonia, (ii) presence of oxygen, (iii) presence of ADP etc. It is because the genes responsible for nitrogen fixation are switched on or off by these compounds. These genes ultimately regulate the nitrogenase synthesis as well as its activity. The mechanism of genetic regulation of nitrogenase is well studied in *Klebsiella pneumoniae*.

The enzyme and its regulatory proteins are encoded by a large number of genes. These genes are organized in several operons that are located close to each other. Together these operons are called as *nif* cluster. The activity of these genes is under the control of *nif LA* operon. This operon, in turn, is regulated by *ntr A* and *C* genes. The product of *nif A* i.e. *nif A* protein acts as a positive regulator. It allows transcription of other genes leading to the synthesis of nitrogenase.

In the presence of ammonia, oxygen etc, *nif L* gene product interacts with *nif A* protein. This results in preventing the activation of *nif* genes and thus the synthesis of nitrogenase. Regulation at genetic level has been found to vary among microorganisms.
**Nitrification**

When organic nitrogen compounds such as amino acids, nucleotides undergo decomposition, ammonia is produced. The process is called as ammonification. The ammonia, then, either gets assimilated (called ammonia assimilation) or gets oxidized to nitrate by a process called nitrification. The bacteria carrying out nitrification are called as nitrifying bacteria and were first isolated by Sergei N. Winogradsky (1856-1953). They are aerobic chemolithotrophs and obtain energy from this oxidation process. Nitrification occurs in two steps:

(i) Ammonia is converted to nitrite by *Nitrosomonas, Nitrosospira, Nitrosococcus*, etc.

(ii) Nitrite is converted to nitrate by *Nitrobacter, Nitrococcus, Nitrospira*, etc.

\[
\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^- 
\]

Nitrification process is very important. In soil, ammonium ions are tightly bound to the clay particles. Therefore, they can’t be taken up by the plants. The nitrifying bacteria convert these ions into nitrate. It can, then, be easily taken up by the plants through the roots and assimilated into organic compounds. At the same time, nitrate is highly water soluble. It is rapidly leached from soils. Hence, nitrification is not beneficial for agriculture purposes.

**Nitrate reduction**

The nitrate produced during various stages undergoes reduction by two types of metabolic process:

(i) Dissimilative metabolism

(ii) Assimilative metabolism

*Dissimilative Metabolism of nitrate*

In anaerobic environment nitrate acts as the final electron acceptor at the end of the electron transport chain and gets reduced to products like \( \text{NO}_2^- \), \( \text{N}_2 \) etc. It is a dissimilatory process.

Some bacteria like *E.coli* can reduce nitrate to nitrite only, by the enzyme nitrate reductase. The process is called dissimilatory nitrate reduction.

\[
\text{NO}_3^- + 2\hat{e} + 2 \text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O} 
\]

As is evident from the equation, nitrate accepts only two electrons to get converted to nitrite producing lesser ATP. Thus, a large amount of nitrate is required for growth. Also, the nitrite formed is quite toxic.

Most of the bacteria completely reduce nitrate to nitrogen gas as follows:

\[
2\text{NO}_3^- + 12\hat{e} + 12 \text{H}^+ \rightarrow \text{N}_2 + 6\text{H}_2\text{O} 
\]

Here nitrate accepts five electrons producing more ATP. Even the product is non-toxic. This process is called as denitrification.

Denitrification is a multistep process and requires four enzymes:

(i) Nitrate reductase – It is a molybdenum – containing membrane bound enzyme.

(ii) Nitrite reductase – In *Paracoccus* and *Pseudomonas*, it contain cyt c and cyt d1. In *Alcaligenes* it is a copper protein. It in present is periplasm of gram–ve bacteria.

(iii) Nitric oxide reductase – It is the membrane bound cyt bc1 complex.

(iv) Nitrous oxide reductase – It is present in periplasm.
Nitrate (NO$_3^-$) $\rightarrow$ Nitrite (NO$_2^-$) $\rightarrow$ Nitric Oxide (NO) $\rightarrow$ Nitrous Oxide (N$_2$O) $\rightarrow$ Dinitrogen (N$_2$)

Denitrification is usually carried out by heterotrophs e.g. some members of the genera *Pseudomonas, Paracoccus, Bacillus, Alcaligenes* etc. These are facultative anaerobes.

Under aerobic conditions, they perform normal respiration. Since the enzymes involved in denitrification are repressed by oxygen, they use this as an alternative process under anaerobic conditions only.

Denitrification is the major process by which gaseous nitrogen is formed biologically. Further, all the products of denitrification are gaseous; they are lost from the environment. This results in loss of soil nitrogen, thereby, affecting the soil fertility.

Assimilative metabolism of nitrate

When nitrate is reduced for use as a nutrient source i.e. it is converted into cell material, the process is called assimilatory nitrate reduction. Unlike dissimilatory process, here nitrate does not participate in energy generation. The process is used by many bacteria, fungi and algae.

The process of assimilatory nitrate reduction is mediated by two enzyme complexes – assimilatory nitrate reductase and assimilatory nitrite reductase as given below. Both the enzymes are present in the cytoplasm.

\[
\begin{align*}
\text{Nitrate} & \xrightarrow{\text{reductase}} \text{Nitrite} & \xrightarrow{\text{reductase}} \text{Ammonia} \\
\ce{NO3^-} & \xrightarrow{\text{reductase}} \ce{NO2^-} & \xrightarrow{\text{reductase}} \ce{NH3} \\
2\bar{e} & \xrightarrow{\text{reductase}} \ce{HNO} & 2\bar{e} \xrightarrow{\text{reductase}} \ce{NH2OH}
\end{align*}
\]

It is clear from these reactions that the first step is the reduction of nitrate to nitrite by nitrate reductase, an enzyme that contains both FAD and molybdenum. NADPH is the source of electrons.

\[
\ce{NO3^- + NADPH + H^+ \rightarrow NO2^- + NADP^+ + H2O}
\]

Nitrite is then reduced to ammonia by nitrite reductase. The ammonia is then incorporated into amino acids.

Ammonia assimilation

Nitrogen is a major component of nucleic acids, proteins and many other cell constituents. Therefore, it is important to assimilate inorganic nitrogen. The sources of nitrogen are atmospheric nitrogen gas, NO$_3^-$ and ammonia. Only a limited number of microorganisms can reduce the gas (Nitrogen Fixation) and use it as a nitrogen source. Some of them use nitrate as nitrogen source by the above discussed dissimilatory nitrogen reduction. However, majority of the microorganisms incorporate ammonia.

Ammonia gets easily incorporated into various organic compounds. This is because nitrogen atom in ammonia is at the same oxidation level as that of the organic nitrogenous
compounds. The route of ammonia assimilation varies from one organism to another. It depends on the enzymes present to carry out the process. Many bacteria and fungi form glutamate by incorporating NH₃ into α-ketoglutarate (a TCA cycle intermediate) by the following reaction:

\[
\text{α-ketoglutarate} + \text{NH}_4^+ + \text{NAD(P)H} + \text{H}^+ \rightarrow \text{Glutamate} + \text{NAD (P)} + \text{H}_2\text{O}
\]

The enzyme glutamate dehydrogenase (GDH) functions at high concentrations of ammonia. At low concentrations of NH₃, ammonia assimilation involves two enzymes, acting in sequence. They are glutamine synthetase (GS) and glutamate synthase (GOGAT).

\[\text{G.S.} \quad \text{L-Glutamate} + \text{NH}_4^+ + \text{ATP} \rightarrow \text{L-Glutamine} + \text{ADP} + \text{Pi}\]

\[\text{GOGAT} \quad \text{L-Glutamine} + \text{α-ketoglutarate} + \text{NADPH} + \text{H}^+ \rightarrow 2[\text{L-Glutamate}] + \text{NADP}^+\]

Some microbes have both the enzyme systems i.e. GDH and GS-GOGAT e.g. *Saccharomyces cerevisiae* and *Bacillus polymyxa*. Some have GDH only e.g. *Streptococcus bovis*, *S. nutans*, *Rhodospirillum purpureus*.

Besides these enzymes, other enzymes involved in ammonia assimilation in some organisms are alanine dehydrogenase e.g. in *rhizobia* and aspartase e.g. *Klebsiella aerogenes*. They catalyze following reactions:

\[\text{Pyruvate} + \text{NH}_4^+ + \text{NAD (P)} + \text{H}^+ \rightarrow \text{L-alanine} + \text{NAD (P)} \]

\[\text{Fumarate} + \text{NH}_4^+ \rightarrow \text{L-aspartate}\]

The amino group of the glutamate and alanine is then transferred to other carbon compounds by transamination reactions. The enzymes involved in such reactions are called transaminases.

\[\text{Glutamate} + \alpha\text{-keto acid} \rightarrow \text{Amino acid} + \alpha\text{-Ketoglutarate.}\]

**Regulation of metabolism**

Micro-organisms have the capability to grow on a variety of substrates. Accordingly, they require different enzymes during growth on various substrates e.g. *E. coli* requires β-galactosidase when growing on lactose but enzyme is not required if glucose serves as a substrate. In addition, the activity of these enzymes has to be under control according to the requirements (energy or cellular constituents) of the organism. Thus, regulation of enzymes is very important aspect of metabolism. The regulation of enzymes occurs at two levels:

1. Regulation of enzyme synthesis. This occurs by two mechanisms – Induction and Repression.
(2) Regulation of enzyme activity. It involves mechanisms like feedback inhibition; allosteric control etc.

Regulation of enzyme synthesis

(i) Induction: The process of substrate mediated enzyme synthesis is termed enzyme induction. The compound turning on the enzyme synthesis is called an inducer e.g. induction of synthesis of β-galactosidase in the presence of lactose. Allolactose, a compound derived from lactose acts as the inducer.

Since metabolism of each substrate requires a number of enzymes, their synthesis can be induced by two mechanisms – coordinate and sequential induction. In coordinate induction, the substrate induces simultaneous synthesis of all the enzymes required for its breakdown. This is found for short catabolic pathways e.g. ED pathway.

Sequential induction is found for long catabolic pathways. These pathways involve degradation of several substrates. Therefore, several inducers are involved e.g. formation of enzymes for the degradation of aromatic compounds in 3-oxoadipate pathway. The difference between the two mechanisms is shown in Fig. 27.

![Fig. 27: Coordinate (a) and sequential (b) induction of enzymes a, b, and c](Source: Bacterial metabolism; G. Gottschalk)

At a given time, a bacterial cell may or may not have the enzyme required for catabolism of a substrate. It means that the entire bacterial genome is not blindly transcribed and translated. The mechanism of induction of enzyme synthesis by inducers is best studied in E.coli for lactose catabolism.

The enzyme β-galactosidase is synthesized only when lactose is present in the culture medium. It has been found that two other proteins are always synthesized along with β-galactosidase. They are galactoside permease and thiogalactoside transacetylase. This shows that genes encoding all the enzymes (and not just one) required by the microbe for getting adapted to a particular environment, are expressed together. Francois Jacob and
Jacques Monod proposed that the expression of these genes is controlled by a common regulator gene and an operator site. Together, they form a unit called operon.

The $lac$ operon consists of a regulator gene, an operator gene and three structural genes encoding enzymes – $\beta$-galactosidase, galactoside permease and thiogalactoside transacetylase. The regulator gene encodes the $lac$ repressor protein. In the absence of lactose, it binds to the operator site. The binding of the repressor to the operator prevents transcription of the structural genes. In the presence of lactose, allolactose is formed. It binds to repressor thereby modifying it. The repressor is now not able to bind to the operator and transcription starts. Hence, synthesis of enzymes (co-ordinate) is induced only in the presence of lactose (Fig. 28). It is thought that a number of inducible enzymes are regulated in similar manner.

![Fig. 28: Diauxic growth of B. subtilis on glucose and arabinose](Source: Bacterial metabolism, G. Gottschalk)

(ii) Catabolite Repression: In 1942, Monod found when a culture of *Bacillus subtilis* was grown in a medium containing both glucose and arabinose, it first utilized glucose and then arabinose. This phenomenon resulted in biphasic growth curve (Fig. 29) and was called as diauxie. Similar results were obtained with a variety of substrate combinations. This meant that the simple substrate inhibited the utilization of complex substrates. Magasanik terms this inhibition as catabolite repression.

The mechanism of catabolite repression has been studied in *E.coli* grown with a mixture of glucose and lactose. The process begins with breakdown of glucose. It causes significant decrease in the intracellular levels of cyclic AMP. After complete utilization of glucose, cAMP levels increase again. cAMP then forms a complex with CRP protein (Cyclic AMP
receptor protein). This complex binds to the lac operon. The process of translation gets initiated.

(iii) End product repression/Attenuation: When a micro-organism grows, some molecules required for an anabolic pathway may be available from the environment. In such cases, enzymes required for biosynthesis of these compounds are not required and their synthesis needs to be stopped. The cell stops the synthesis of these enzymes by end product repression and/or attenuation.

![Diagram of lac operon and end product repression](image)

**Fig. 29:** Induced synthesis of the enzymes for lactose catabolism: a: The product of the gene \( I \) is a repressor protein that binds to the \( O \) region of the lac operon and thus prevents transcription of this operon; b: The inducer (allolactose) binds to the repressor, which thereby loses its affinity to the \( O \) region, mRNA is made, and the three proteins are formed. \( P \), promotor; \( O \), operator; \( Z, Y \), and \( A \), genes coding for \( \beta \)-galactosidase, permease, and transacetylase

(Source: Bacterial metabolism, G. Gottschalk).

The mechanism of end product repression is clearly understood in \( Trp \) operon for the tryptophan synthesis. When tryptophan is present in the environment, it is transported inside the cell. It gets accumulated and combines with repressor protein present in the cell in its inactive form (called as aporepressor). After combining with tryptophan, it becomes an active repressor. It then binds to the operator region and prevents transcription of the operon (Fig. 30).

\( Trp \) operon is also regulated by a unique mechanism called attenuation (the word means “to lessen in amount”). This was discovered by Charles Yanofsky & his colleagues in microorganisms. They found that here regulation did not occur at the operon level but at the transcription and translation stages. The \( Trp \) operon five structural genes, which code for five proteins of the tryptophan biosynthetic pathway. In addition, it has a promoter sequence, an operator sequence and a sequence called leader sequence, present in the
beginning. The leader sequence codes for a tryptophan rich polypeptide which functions as an attenuator.

![Diagram of trp operon and mechanism of end product repression](image)

**Fig. 30: The tryptophan operon and the mechanism of end product repression:** a. If tryptophan does not accumulate an inactive repressor is present; the structural genes are transcribed and proteins are synthesized. b. If tryptophan accumulates an active repressor is formed and transcription is repressed

(Source: Bacterial metabolism, G. Gottschalk)

When tryptophan is present in sufficient concentration in environment, the leader peptide is synthesized. This results in termination of transcription of the entire *trp* operon. However, when tryptophan is in short supply, the synthesis of leader peptide is blocked and transcription of the rest of the operon continues. Let’s see how does translation of the leader peptide regulates transcription of the tryptophan genes? In bacteria, transcription and translation are tightly linked to each other i.e. they occur simultaneously. Thus, as transcription of DNA sequences proceeds, the ribosome immediately binds to the mRNA (Fig. 31). The process of translation begins. Attenuation occurs because a portion of the newly formed mRNA folds into a structure called stem loop. Since this stem loop structure is followed by runs of uracils, it is an effective terminator of transcription. Thus, transcription is stopped before the RNA polymerase reaches the first structural gene of the operon. But when tryptophan is in short supply, the ribosome pauses at a tryptophan codon. The presence of stalled ribosome at this position allows an alternative confirmation of the stem loop. This stem loop is not a terminator structure and transcription continues into structural genes. Similarly, *his* operon is also regulated by attenuation

**Regulation of enzyme activity**

During various biosynthetic reactions, it is important to regulate the amount of product being synthesized. This is done by immediately reducing the synthesis of enzymes involved. Various mechanisms are used for this purpose.
Fig. 31: Control of transcription of tryptophan operon structural genes by attenuation in Escherichia coli. The leader peptide is coded by regions 1 and 2 pf the mRNA. Two regions of the growing mRNA chain are able to form double-stranded loops, shown as 2:3 and 3:4. Under conditions of excess tryptophan, the ribosome translates the complete leader peptide, and so region 2 cannot pair with region 3. Regions 3 and 4 then pair to form a loop that terminates RNA polymerase. If translation is stalled because of tryptophan starvation, loop formation via 2:3 pairing occurs, loop 3:4 does not form, and transcription proceeds past the leader sequence. If the ribosome cannot begin translation of the leader because of some translational block other than tryptophan starvation, then loop 3:4 will be free of form, and transcription is also terminated.

(Source: Brock Biology of microorganisms, Madigan, Martinko & Parker)
(i) **Feedback inhibition:** When the end product is in excess, it inhibits the activity of the first enzyme involved specifically in its formation. e.g. if isoleucine is synthesized in excess, it inhibits the activity of the first enzyme of its biosynthetic pathway.

\[
\text{Threonine} \xrightarrow{\text{Threonine deaminase}} \alpha \text{-oxobutyrate} \rightarrow \rightarrow \rightarrow \text{Isoleucine}
\]

As soon on threonine deaminase is inhibited, the subsequent enzymes of this pathway do not get the substrates. Hence, the synthesis of the end product stops. The enzymes undergoing feedback inhibition are called as allosteric enzymes. They have a property known as allostery. An allosteric enzyme has two important binding sites:

(a) the **active site** where the substrate binds. This site is specific for the substrate.

(b) the **allosteric site** where the specific inhibitor binds noncovalently and reversibly.

The inhibitors are generally small metabolites or cofactors, and are also called allosteric modulators or allosteric effectors. When an inhibitor binds at the allosteric site, the confirmation of the enzyme molecule changes (Fig. 32). As a result, the substrate can’t bind efficiently at the active site. When the concentration of inhibitor falls, equilibrium favours dissociation of the inhibitor from the allosteric site, returning the enzyme molecule to its original confirmation.

![Fig. 32: Mechanism of enzyme inhibition by an allosteric effector. When the effector combines with the allosteric site, the conformation of the enzyme is altered to that the substrate can no longer bind](Source: Brock Biology of Micro-organism, Madigan, Martinko & Parker)
(ii) **Cova lent modification of enzymes:** The activity of some enzymes gets modified by covalent attachment of a molecule. The attached molecules provide a functional group that modifies the properties of the enzyme. Some of the common methods of covalent modification are phosphorylation and dephosphorylation, adenylation/deadenylation, acetylation/deacetylation etc.

\[
\text{Enzyme} - \alpha \quad \text{Enzyme} + \alpha
\]

(Enzyme – α (active/inactive) \quad \text{Enzyme} + \alpha (inactive/active))

Most of these modifications are reversible. These are more common among eukaryotes. In bacteria, isocitrate dehydrogenase of *E. coli* and *Salmonella typhimurium* is active in dephosphorylated form. However, on phosphorylation it becomes inactive.

It can be concluded that bacteria use various mechanisms for regulation of both enzyme synthesis and enzyme activity. These mechanisms vary from micro-organism to micro-organism.

**Suggested Readings**

1. Microbial Physiology by Moat, Foster & Spector
2. Bacterial Metabolism by Gerhard Gottschalk
3. Microbiology by Prescott, Harley & Klein
4. Biochemistry by L. Stryer
5. Concepts in Biochemistry by Rodney Boyer
7. Microbial life by Perry, Staley & Lory.