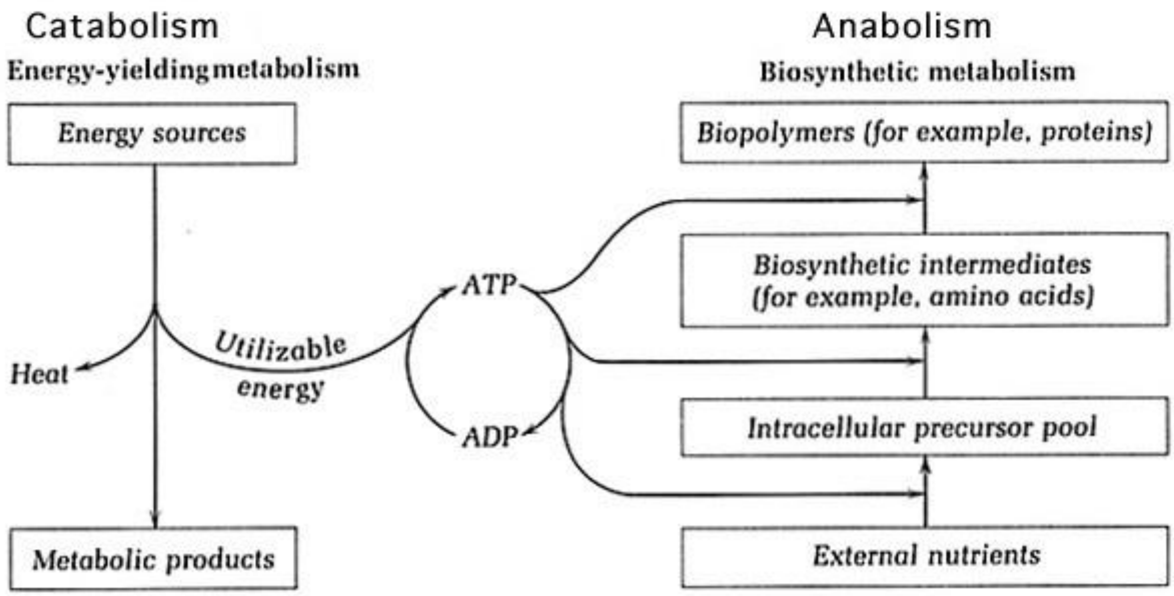
14
BACTERIAL METABOLISM

14.1. ENERGY-GENERATING METABOLISM

The term **metabolism** refers to the sum of the biochemical reactions required for energy generation and the use of energy to synthesize cell material from small molecules in the environment. Hence, metabolism has an **energy-generating component**, called **catabolism (breakdown of molecules)**, and **an energy-consuming**, **biosynthetic component**, called **anabolism**. Catabolic reactions or sequences produce energy as **ATP**, which can be utilized in anabolic reactions to build cell material from nutrients in the environment.



The relationship between catabolism and anabolism in a cell. During catabolism, energy is changed from one form to another, and keeping with the laws of thermodynamics, such energy transformations are never completely efficient, i.e., some energy is lost in the form of heat. The efficiency of a catabolic sequence of reactions is the amount of energy made available to the cell (for anabolism) divided by the total amount of energy released during the reactions.

14.2. BASIC CONCEPTS OF METABOLISM

Before going into the microbial metabolism, it is essential to understand the basics related to it.

14.2.1. ENERGY

It is defined as the **capacity to do work**. The energy can be referred as kcal or K Joules. One kcal is the energy required to raise 1°C of 1 kg of water. 1 calorie = 4.184 Joules. Most of the cells obtain energy by carrying out the chemical reactions viz., oxidation of organic or inorganic compounds which liberate energy. The energy released during oxidation or reduction reaction is called **chemical energy**.

The living things should take the energy from existing sources and convert them to a suitable form for the biological process. Plants absorb the energy from light and convert them to chemical compounds of high energy. Similarly, animals can derive their energy by oxidizing the chemicals. Microorganisms especially the bacteria have both the ways (from chemicals and sun light)

In any chemical reaction, energy will be liberated or absorbed. The released energy is called **total energy**.

14.2.2. FREE ENERGY (ΔG)

Free energy (ΔG) is defined as the part of the total energy which is **available** for the performance of some useful work.

14.2.3. ENTROPHY

Entrophy is a measure of the part of the total energy which is **unavailable** for the useful work.

Total energy = free energy (ΔG) + entropy

14.2.4. EXERGONIC & ENDERGONIC REACTIONS

A reaction may release or require free energy. If a reaction **needs energy**, it is referred as endergonic reaction and if a reaction **releases energy**, it is referred as exergonic reaction.

If ΔG of a chemical reaction has a **negative value** (- 5000 cal), the reaction is exergonic reaction. If ΔG of a chemical reaction has a **positive value** (2500 cal), the reaction requires energy (ie. Endergonic reaction).

A + B → C + D + energy [exergonic reaction]

A + B + energy → C + D [endergonic reaction]

ΔG of some compounds are,

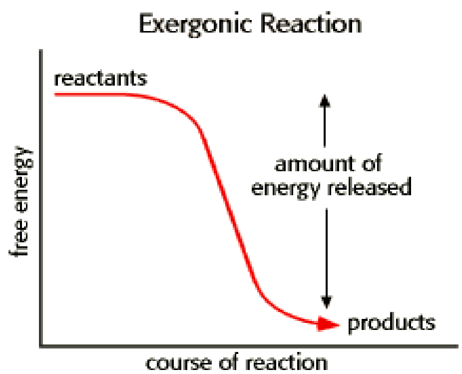
H_20 = -237.2;

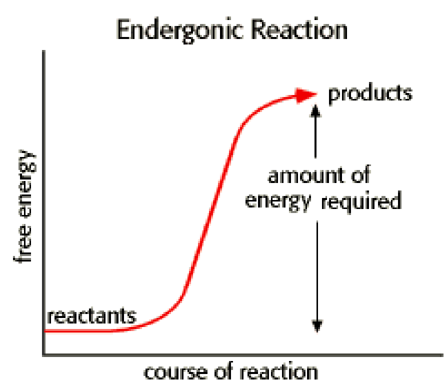
CO_2 = -396.4;

H_2 = 0;

O_2 = 0;

N_2O = +104.2;





Glucose = -917.3

Negative ΔG is good and available to do work in the cell.

14.2.5. ACTIVATION ENERGY

It is referred as the energy required to **bring** all the **molecules to reactive stage**. When the H_2 and O_2 gases kept together in a container will not react each other even after many years and need some energy to make them as reactive molecules to react and gives H_2O. That energy is referred as activation energy.

14.2.6. CATALYST

Catalyst is a **substance to lower the activation energy** requirement of a reaction and also to increase the reaction rate.

In biological systems, proteins involved in the catalytic activity are referred as **biocatalysts** or **enzymes**. They increase the reaction rate of 10^8 to 10^{20} times.

14.2.7. ENZYMES

Enzymes are **protein** in nature with the size of 1x 10^4 to 1 x 10^6 Dalton (1 Dalton =1.67 x 10^{-24} g). Normally some non-proteinaceious, low molecular substances were joint together with protein part to serve as enzymes. Enzymes are very specific, efficient and can be inhibited by products.

$$\text{Protein + Non-protein compounds} \rightarrow \text{enzyme}$$

The protein part is **Apoenzyme.** The non-protein is referred as **Co-enzyme** or **Prosthetic group.** Both (apoenzyme and coenzyme / prosthetic group) are referred as **Holoenzymes**. The difference between co-enzyme and prosthetic group is that co-enzyme will have loose bound with apoenzyme (temporarily) whereas the prosthetic group will have tight bound with apoenzymes (permanently).

For Example,

- **Co-enzymes** – NADP, NAD, FAD, FAM
- **Prosthetic group** – Heme group of cytochromes

Apart from the co-enzymes and prosthetic groups, the inorganic ions like Ca, Mg, Fe, Mn are required for the activation of enzyme and they are referred as **co-factors.**

14.2.8. ROLE OF ATP IN METABOLISM

Energy is released from a cell in an exergonic reaction (ie. The reactions with $-\Delta G$). Instead of wasting this energy, much of it is trapped and used for doing work (endergonic reactions). In living organisms this practical form of energy is **adenosine triphosphate** (ATP). In a sense, cells carry out certain processes so that they can earn ATP and carry out other processes in which they spend their ATP. Thus, ATP is referred to as the cell's energy currency.

ATP serves as the link between exergonic reactions and endergonic reactions. ATP is a high energy molecule. It breaks down or hydrolyses completely to the products adenosine diphosphate (ADP) and orthophosphate + energy. This energy is used to resynthesize ATP from ADP and P_i during catabolism. Thus, **ATP, ADP** and **P_i** form an energy cycle.

14.2.9. OXIDATION – REDUCTION REACTION

Several types of chemical reactions are involved in energy production. Oxidation – reduction reaction is the common method to derive energy by the living cells.

OXIDATION AND REDUCTION

Oxidation is the loss of electrons and **reduction** is the gain of electrons. Oxidation reduction reactions are dehydrogenations (reaction involves the loss of hydrogen atoms). (Note: Remember the phrase "LEO says GER" LEO – **L**oss of **E**lectron is **O**xidation; GER – **G**ain of **E**lectron is **R**eduction)

OXIDIZING AGENT

An oxidizing agent **(oxidant)** will absorb electrons and will therefore become reduced, as illustrated by the following examples.

The ferric ion is an oxidizing agent; it absorbs electrons and becomes reduced to ferrous ion:

$$\underset{\text{Ferric ion}}{Fe^{3+}} + \underset{\text{Electron}}{e^-} \rightarrow \underset{\text{Ferrous ion}}{Fe^{2+}}$$

REDUCING AGENT

A reducing agent **(reductant)** donates electrons, becoming oxidized in the process. The ferrous ion is a reducing agent, it donates electrons and becomes oxidized to ferric ion:

$$\underset{\text{Ferrous ion}}{Fe^{2+}} \rightarrow \underset{\text{Ferric ion}}{Fe^{3+}} + \underset{\text{Electron}}{e^-}$$

Here, the Fe^{3+} accepts electrons and hence referred as **electron acceptor**. Fe^{2+} donates the electron and is referred as **electron donor**.

OXIDATION - REDUCTION POTENTIAL

Substrates or compounds vary their ability to donate or accept the electrons (in other words, to become oxidized or reduced). The tendency of a substrate to donate or accept the electron is referred as oxidation – reduction Potential or **redox potential.** The redox potential of each substance was calculated by means of electrical volt with reference substance as H_2. The redox potential is –ve means, the substance is rich of electrons, (means reduced form) and +ve means it already lost the electrons (means oxidized form). The reduced forms can give or donate electrons and oxidized form can accept the electrons.

ELECTRON CARRIERS

In a series of oxidation-reduction reactions, the electron from one reaction can be carried out to another through some carriers called **electron carriers.** Ex: NAD, NADP, FAD, FAM, cytochromes, etc.

14.3. ENERGY PRODUCTION IN BACTERIA

Energy production in bacteria occurs by three ways. They are as follows:

a. Respiration

b. Fermentation

c. Phosphorylation

RESPIRATION

An energy yielding process in which the energy substrate is oxidized using the exogenous or externally derived electron acceptor. Respiration may be aerobic or anaerobic.

FERMENTATION

An energy yielding reaction in which an organic molecule is oxidized with out an exogenous electron acceptor. Usually pruvate or pyruvate derived substrates act as electron acceptors.

14.3.1. PHOSPHORYLATION

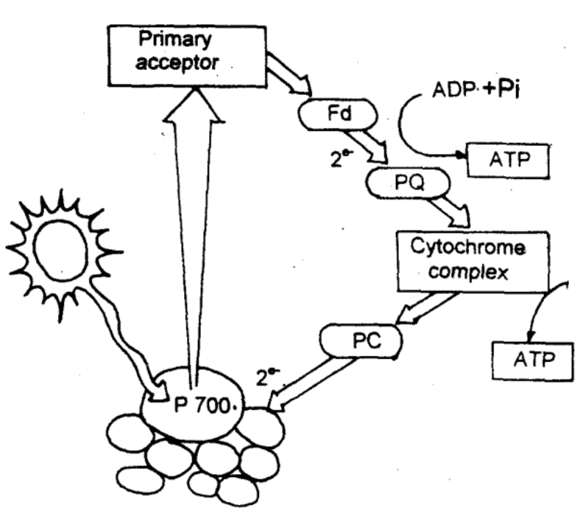
Addition of a PO_4 group to a compound is called phosphorylation. The energy released during oxidation reaction is stored in the form of **high energy phosphate bonds** in ATP. When energy is released, the ADP and Pi reacted and form ATP. This process of addition of Pi to from ATP is referred as phosphorylation.

Three types of phosphorylation occurs through which the ATP formation occurs in bacteria.

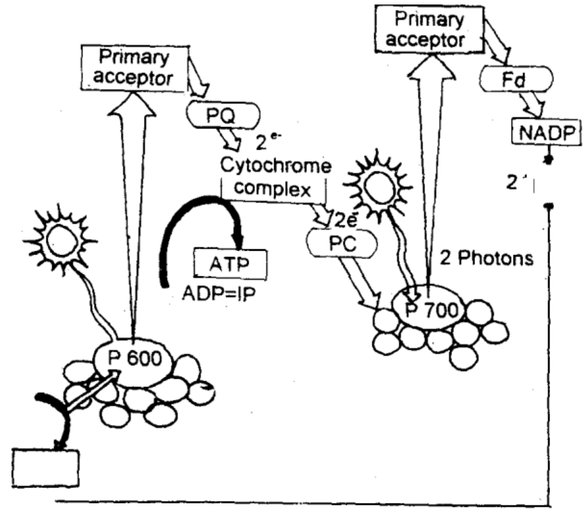
a. **SUBSTRATE LEVEL PHOSPHORYLATION** - During the oxidation of one organic molecule, energy will be released, which can be used for the production of ATP. The organic substrate itself acts as electron acceptor. This kind of phosphorylation is referred as **substrate level phosphorylation.**

b. **OXIDATIVE PHOSPHORYLATION** - During electron transport chain, a series of oxidation – reduction reactions occur in cells. These reactions are mediated by a number of enzymes. As the electron flows through the chains, much of their free energy is conserved in the form of ATP. This process is called oxidative phosphorylation. It is also referred as **electron transport phosphorylation**.

c. **PHOTOPHOSPHORYLATION –** Utilization of light energy to derive the synthesis of ATP is referred as phosphorylation. The proton motive force during photosynthesis is used for ATP synthesis.



Cyclic photophosphorylation



Non cyclic photophosphorylation

14.3.2. ergy production in Heterotrophs / Heterotrophic Metabolism

Heterotrophic metabolism is directed by two metabolic processes. They are,

a. **Fermentation** and

b. Respiration

Heterotrophs are the microorganisms that are unable to use carbondioxide as its sole source carbon and requires one or more organic compounds. Heterotrophic bacteria can use a variety of organic compounds as energy sources. The compounds include carbohydrates, fatty acids and amino acids. The most preferred compounds are carbohydrates especially 6 carbon sugar – **Glucose**.

Glucose can be taken up by bacterium through diffusion and can be readily utilized. The bacteria can use glucose by following these pathways.

a. Embden Meyerhof pathway
b. Phosphoketolase pathway
c. Entner - Doudoroff pathway

These three pathways end with one or two ATP synthesis by **substrate level phosphorylation**.

a) EMBDEN – MEYERHOF PATHWAY

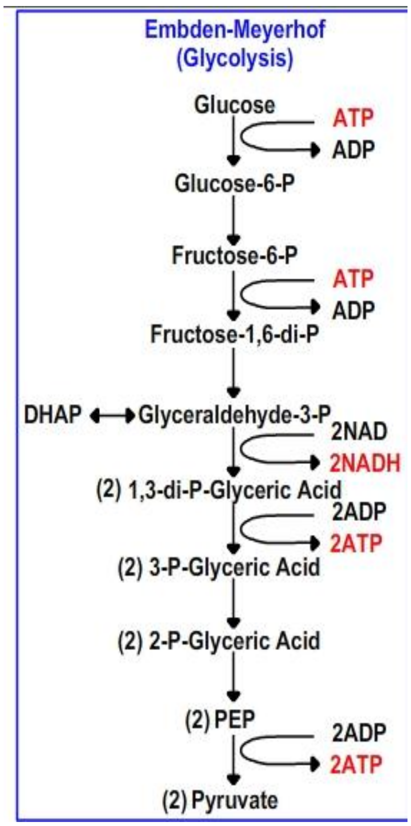
The most common pathway of glucose catabolism is the Embden-Meyerhof pathway of **glycolysis** (“splitting of glucose”). This process occurs very widely and is found in microorganisms as well as in animals and plants. Glycolysis does not require the presence of oxygen and therefore can occur in both aerobic and anaerobic cells. Aerobic cells degrade glucose by glycolysis, and this process constitutes the preparatory stage for aerobic phase of glucose oxidation.

Thus, under **anaerobic conditions** this situation prevails:

$$\text{Glucose} \xrightarrow{\text{fermentation}} \text{fermentation products}$$

Whereas under **aerobic conditions**, the following occurs:

$$\text{Glucose} \xrightarrow{\text{fermentation}} \text{intermediate} \xrightarrow{\text{respiration}} CO_2 + H_2O$$



This pathway operates in yeast to produce alcohol and in lactic acid bacteria to produce lactic acid and other organic acids, fatty acids and alcohols.

The overall reaction of EMP of glycolysis is as follows:

$$\mathbf{C_6H_{12}O_6 + 2NAD + 2ATP + 2Pi \rightarrow 2CH_3COCOOH + 2NADH_2 + 2ATP}$$

Glucose **Pyruvic acid**

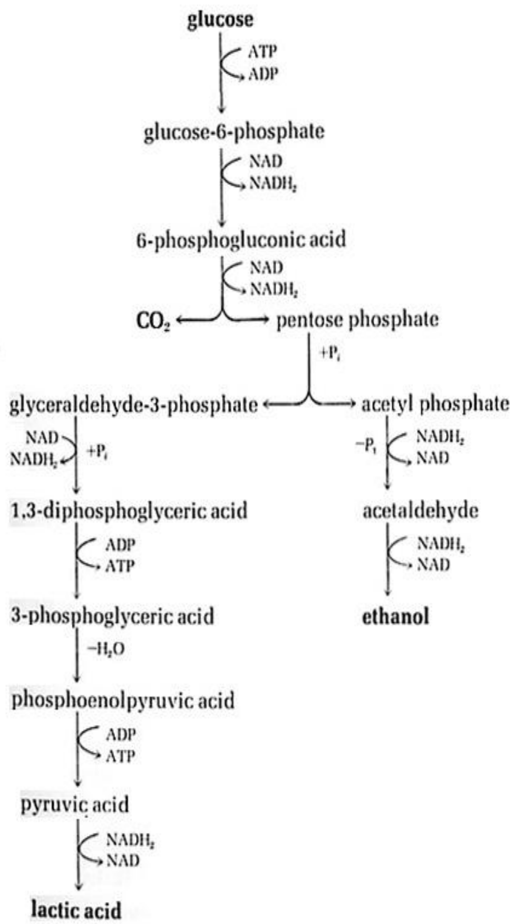
After pyruvate formation, if the organism is aerobic / respiratory type, it enters into the Kreb's cycle and if the organism is fermentative / anaerobic, the reduction process occurs which ends with the production of organic acids / alcohols, etc. Complete oxidation leads to respiration and the reduction process leads to fermentation.

The Embden – meyerhof path way can lead to a wide array of end products depending on the path ways taken in the reductive steps after the pyruvate formation. Based on the fermentative products formed , the pathway has been named .

The following are some of such fermentations:

Fermentation	**End products formed form pyruvate**	**Model organism**
Homolactic fermentation	Lactic acid	*Lactobacillus*
Mixed acid fermentation	Lactate, acetate, formate, succinate	*Enterobater*
Butyric acid fermentation	Butric acid, acetone	*Clostridium acetobutylicum*
Propionic acid fermentation	Propionic acid	*Propionibacterium*
Alcohol fermentation	Ethanol	*Saccharomyces*

b) PHOSPHOKETOLASE PATHWAY / HETEROLACTIC PATHWAY



The phosphoketolase path way is distinguished by the key cleavage enzyme **phosphoketolase,** which cleaves pentose to glyceroldehyde 3 phosphate and acetyl phosphate. The path way ends with ethanol and lactic acid. Ex. *Lactobacillus, Leuconostoc.*

The overall reaction is

Glucose → 1 lactate + 1 ethanol + 1 CO_2 + 1 ATP

This path way is useful in the dairy industry for preparation of kefir (fermented milk), yogurt, etc.

c) ENTNER - DOUDOROFF PATHWAY

This pathway is found in both aerobic and anaerobic prokaryotes but **not in eukaryotes**. It is widespread among soil microbes such as *Pseudomonas, Azotobacter*, *Agrobacterium* and a few Gram negative bacterium, very few Gram positive have this pathway (*Enterococcus faecalis*).

Here, glucose is phosphorylated to glucose-6-phosphate. It is then oxidized to 6-phosphogluconic acid. A dehydration step follows to yield 2-keto-3-deoxy-6-phosphogluconic acid (**KDPG**); the latter is cleaved to pyruvic acid and glyceraldehydes-3-phosphate, which is metabolized via some EMP pathway enzymes to produce a second molecule of pyruvic acid. In the aerobic pseudomonads, the catabolism is completed via acetyl - CoA and the tricarboxylic acid cycle.

This pathway will be operating in *Zymomonas mobilis* by converting pyruvate to ethanol.

Glucose → 2 ethanol + 2 CO_2 + 1 ATP