Unit – 3 Metabolism, Bioenergetics

- **Metabolism:**

Metabolism is the set of life-sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments. The word metabolism can also refer to all chemical reactions that occur in living organisms, including digestion and the transport of substances into and between different cells.

Metabolic networks have three major functions:

i. to extract energy from nutrients or solar energy;
ii. to synthesize the building blocks that make up the large molecules of life: proteins, fats, carbohydrates, nucleic acids, and combinations of these substances;
iii. to synthesize and degrade molecules required for special functions in the cell.

The chemical reactions of metabolism are organized into metabolic pathways, in which one chemical is transformed through a series of steps into another chemical, by a sequence of enzymes. Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy that will not occur by themselves, by coupling them to spontaneous reactions that release energy. Enzymes act as catalysts that allow the reactions to proceed more rapidly. Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or to signals from other cells.

Most of the structures that make up animals, plants and microbes are made from three basic classes of molecule: amino acids, carbohydrates and lipids (often called fats). As these molecules are vital for life, metabolic reactions either focus on making these molecules during the construction of cells and tissues, or by breaking them down and using them as a source of energy, by their digestion. These biochemicals can be joined together to make polymers such as DNA and proteins, essential macromolecules of life.

Metabolism is usually divided into two categories: catabolism and anabolism.

- **Catabolism:**

Catabolism is the set of metabolic pathways that breaks down molecules into smaller units that are either oxidized to release energy, or used in other anabolic reactions. Catabolic reactions are used to capture and save energy from nutrients, as well as to degrade larger molecules into smaller, molecular raw materials for reuse by the cell. The energy is stored in the form of energy-rich ATP, whichpowers the reactions of anabolism.

Catabolism breaks down large molecules (such as polysaccharides, lipids, nucleic acids and proteins) into smaller units (such as monosaccharides, fatty acids, nucleotides, and amino acids, respectively). As molecules such as polysaccharides, proteins, and nucleic acids comprise long chains of these small monomer units, the large molecules are called polymers.

Cells use the monomers released from breaking down polymers to either construct new polymer molecules, or degrade the monomers further to simple waste products, releasing energy. Cellular wastes include lactic acid, acetic acid, carbon dioxide, ammonia, and urea. The creation of these wastes is usually an oxidation process involving a release of chemical free energy, some of which is lost as heat, but the rest of which is used to drive the synthesis of adenosine triphosphate (ATP).

- **Anabolism**:

- It is the set of metabolic pathways that construct molecules from smaller units.
- **Anabolism** is the set of constructive metabolic processes where the energy released by catabolism is used to synthesize complex molecules.
- Anabolism involves three basic stages. Firstly, the production of precursors such as amino acids, monosaccharides,isoprenoids and nucleotides, secondly, their activation into reactive forms using energy from ATP, and thirdly, the assembly of these precursors into complex molecules such as proteins, polysaccharides, lipids and nucleic acids.
- Anabolism uses energy stored in the form of adenosine triphosphate (ATP) to build larger molecules from smaller molecules.

These reactions require energy. Anabolic processes tend toward "building up" organs and tissues. These processes produce growth and differentiation of cells and increase in body size, a process that involves synthesis of complex molecules. Examples of anabolic processes include the growth and mineralization of bone and increases in muscle mass.

- **Free energy**

Free energy (G) is the amount of energy available to do work under the conditions of a biochemical reaction.

The multiplicity of processes performed by all biological systems can be traced, directly or indirectly, to certain chemical reactions. The term metabolism denotes all the organized chemical activities performed by a cell, which compromise two general type- energy production and energy utilization.

Most cells obtain energy by carrying out chemical reactions which liberate energy. In the course of any chemical reaction, energy available for the performance of useful work is either released or absorbed. The amount of energy liberated or taken up during the course of a reaction is referred to as the free energy change (ΔG) of the reaction. It is expressed in terms of calories. If the (ΔG) of a chemical reaction has negative value, the reaction releases energy and is termed as exergonic reaction. If the (ΔG) has positive value, the reaction requires energy and termed as endergonic reaction. Concentration of reactants affects the value of (ΔG) for a chemical reaction. The concentration of all reactants at 1M in the steady state; referred to as standard concentration.

Under conditions of standard concentration, the free energy change (ΔG) of a reaction is referred to by a special term, (ΔG^0). In other words, (ΔG^0) is the amount of free energy released or absorbed when one mole of the reactant is converted to one mole of product at $25\ ^0C$ and one atmosphere of pressure, and under (hypothetical) conditions, where all reactants and products are maintained at 1M concentration.

The (ΔG^0) or standard free energy change is related to the equilibrium constant $K_{eq,}$ of a chemical reaction by the equation.

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❖ Bioenergetics

Bioenergetics is a field in biochemistry that concerns energy flow through living systems. This is an active area of biological research that includes the study of thousands of different cellular processes such as cellular respiration and the many other metabolic processes that can lead to production and utilization of energy in forms such as ATP molecules.

Bioenergetics is the part of biochemistry concerned with the energy involved in making and breaking of chemical bonds in the molecules found in biological organisms. It can also be defined as the study of energy relationships and energy transformations in living organisms.

Growth, development and metabolism are some of the central phenomena in the study of biological organisms. The role of energy is fundamental to such biological processes. The ability to harness energy from a variety of metabolic pathways is a property of all living organisms. Life is dependent on energy transformations; living organisms survive because of exchange of energy within and without.

In a living organism, chemical bonds are broken and made as part of the exchange and transformation of energy. Energy is available for work (such as mechanical work) or for other processes (such as chemical synthesis and anabolic processes in growth), when weak bonds are broken and stronger bonds are made. The production of stronger bonds allows release of usable energy.

Living organisms produce ATP from energy sources via oxidative phosphorylation. The terminal phosphate bonds of ATP are relatively weak compared with the stronger bonds formed when ATP is broken down to adenosine monophosphate and phosphate and then dissolved in water. Here it is the energy of hydration that results in energy release. An organism's stockpile of ATP is used as a battery to store energy in cells, for intermediate metabolism. Utilization of chemical energy from such molecular bond rearrangement powers biological processes in every biological organism.

- **Exergonic** is a spontaneous reaction that releases energy. It is thermodynamically favored. On the course of a reaction, energy needs to be put in, this activation energy drives the reactants from a stable state to a highly energetic unstable configuration. These reactants are usually complex

molecules that are broken into simpler products. The entire reaction is usually catabolic. The release of energy (called Gibbs free energy) is negative and equal to - ΔG because energy is lost from the bonds formed by the products.

- **Endergonic** is an anabolic reaction that consumes energy. It has a positive ΔG because energy is required to break bonds.

The free energy (ΔG) gained or lost in a reaction can be calculated:

$$\Delta G = \Delta H - T\Delta S$$

where G = Gibbs free energy, H = enthalpy, T = temperature, and S = entropy.

❖ **Chemical links between catabolism and biosynthesis (anabolism)**

Catabolic and anabolic pathways are interrelated in many ways:

i. Matter (catabolic pathways furnish the precursor compounds for anabolism.
ii. Energy (catabolic pathways furnish the energy to "drive" anabolism).
iii. Electrons (catabolic pathways furnish the reducing power for anabolism).
iv. Linear pathways convert one compound through a series of intermediates to another compound. An example would be glycolysis, where glucose is converted to pyruvate.
v. Branched pathways may either be divergent (an intermediate can enter several linear pathways to different end products) or convergent (several precursors can give rise to a common intermediate). Biosynthesis of purines and of some amino acids are examples of divergent pathways. There is usually some regulation at the branch point. The conversion of various carbohydrates into the glycolytic pathway would be an example of convergent pathways.
vi. In a cyclic pathway, intermediates are regenerated, and so some intermediates act in a catalytic fashion. In this illustration, the cyclic pathway carries out the net conversion of X to Z. The Tricarboxylic Acid Cycle is an example of a cyclic pathway.
vii. A pool of compounds in equilibrium with each other provides the intermediates for converting compounds to a variety of products, depending on what is fed "into" the pool and what is "withdrawn" from the pool.

- **Energy coupling through ATP and pyridine nucleotides**

Energy Coupling - the use of an exergonic process to drive and endergonic process . The free energy released from the exergonic process is absorbed by the endergonic process.

OXIDATION – REDUCTION REACTIONS

Oxidation is the loss of electrons; reduction is the gain of electrons. Frequently, oxidation reactions are dehydrogenations (reactions involving the loss of hydrogen atoms); since a hydrogen atom consists of a proton plus an electron, a compound which loses a hydrogen atom has essentially lost an electron and therefore has been oxidized. An oxidizing agent (oxidant) will absorb electrons and will therefore become reduced. Eg: Fumaric acid is an oxidizing agent; it absorbs hydrogen atoms (which contain electrons) and becomes reduced to succinic acid. A reducing agent (reductant) donates electrons, becoming oxidized in the process. One can notice that the reverse of each oxidation reaction is a reduction and the reverse of a reduction reaction is an oxidation. Eg: succinic acid conversion to fumaric acid. Pair of substances which involves in such type of reaction is referred to as oxidationreduction (O/R) system.

One O/R system may tend to absorb electrons from another O/R system; i.e; the first system will oxidize the second. On the other hand, the tendency of the first system to absorb electrons may be so low that the second system may oxidize the first. This power (the tendency to absorb electrons) is expressed by the standard oxidation – reduction potential or the electromotive potential (E'o) of an O/R system, which is measured electrically under standardized conditions of comparison (electron donor and its conjugate at 1M concentration, 25^0 C, and pH 7) and expressed in volts. The more positive the E'o, the greater the oxidizing ability of the system. Such relationships are very important in understanding the orderlysequence in which biological oxidations occur.

When one O/R system oxidizes another, energy is released. It is important to know the values of E'o for each system, because the (ΔG^0) of the overall reaction is directly proportional to the difference in E'o values. If the voltage difference is large, an amount of free energy sufficient to drive the synthesis of ATP may be liberated.

In respiration, an oxidizable substrate is the primary electron donor. In aerobic respiration the terminal electron acceptor is oxygen; in anaerobic respiration the final electron acceptor is a compound like fumarate, NO_3^-, SO_4^{2-},

or CO_3^{2-}. In fermentation, an organic compound is the final electron acceptor; an oxidizable substrate is the electron donor. In photosynthesis carried out by bacteria, bacteriochlorophylls serve as both electron donors and acceptors. In photosynthesis by green algae, plants and cyanobacteria, water serves as a primary electron donor and NADP+ as a terminal electron acceptor. The paths through which these electrons flow in the various processes are called electron transport chains.

Electron transport chains are sequences of oxidation-reduction reactions that occur in cells. These reactions are mediated by a number of electron carriers and electron carrier enzymes. As the electrons flow through the chains, much of their free energy is conserved in the form of ATP; this process is called oxidative phosphorylation.

The multicomponent electron-transport chains are always associated with membranes. In eukaryotes, they are in mitochondrial or chloroplast membranes; in prokaryotes, they are in the cytoplasmic membrane

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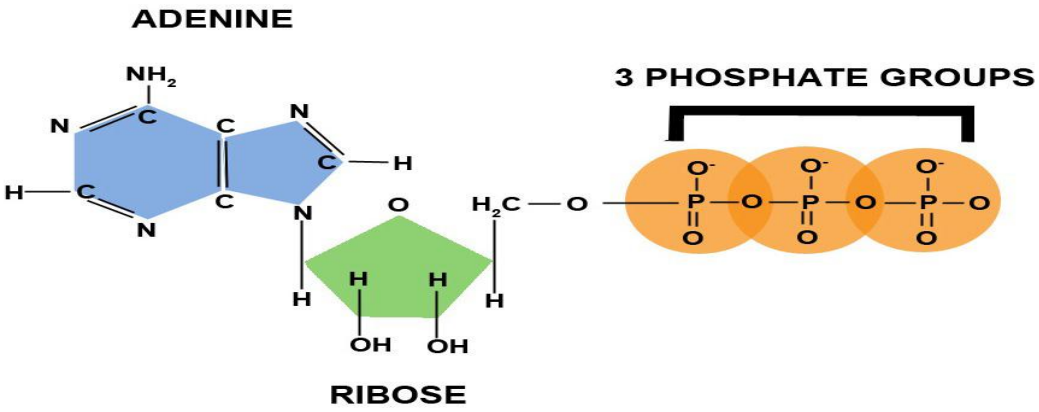
❖ **Adenosine Triphosphate (ATP)**



Adenosine triphosphate (**ATP**) is a nucleoside triphosphate used in cells as a coenzyme often called the "molecular unit of currency" of intracellular energy transfer.

ATP transports chemical energy within cells for metabolism. It is one of the end products of photophosphorylation, cellular respiration, and fermentation and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains three phosphate groups, and it is produced by a wide variety of enzymes, including ATP synthase, from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors.

Substrate-level phosphorylation, oxidative phosphorylation in cellular respiration, and photophosphorylation in photosynthesis are three major mechanisms of ATP biosynthesis.

Metabolic processes that use ATP as an energy source convert it back into its precursors. ATP is therefore continuously recycled in organisms.

The structure of this molecule consists of a purine base (adenine) attached to the 1' carbon atom of a pentose sugar (ribose). Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

ATP consists of adenosine — composed of an adenine ring and a ribose sugar — and three phosphate groups (triphosphate). The phosphoryl groups, starting with the group closest to the ribose, are referred to as the alpha (α), beta (β), and gamma (γ) phosphates. Consequently, it is closely related to the adenosine nucleotide, a monomer of RNA.

Two phosphoanhydride bonds (those that connect adjacent phosphates) in an ATP molecule are responsible for the high energy content of this molecule. In the context of biochemical reactions, these anhydride bonds are frequently—and sometimes controversially—referred to as *high-energy bonds* (despite the fact it takes energy to break bonds). Energy stored in ATP may be released upon hydrolysis of the anhydride bonds. The primary phosphate group on the ATP molecule that is hydrolyzed when energy is needed to drive anabolic reactions is the γ-phosphate group. Located the farthest from the ribose sugar, it has a higher energy of hydrolysis than either the α- or β-phosphate. The bonds formed after hydrolysis—or the phosphorylation of a residue by ATP—are lower in energy than the phosphoanhydride bonds of ATP. During enzyme-catalyzed hydrolysis of ATP or phosphorylation by ATP, the available free energy can be harnessed by a living system to do work.

The standard amount of energy released from hydrolysis of ATP can be calculated from the changes in energy under non-natural (standard) conditions, then correcting to biological concentrations. The net change in heat energy (enthalpy) at standard temperature and pressure of the decomposition of ATP into hydrated ADP and hydrated inorganic phosphate is −30.5 kJ/mol, with a

change in free energy of 3.4 kJ/mol.[17] The energy released by cleaving either a phosphate (P_i) or pyrophosphate (PP_i) unit from ATP at standard state of 1 M are:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^\circ = -7.3 \text{ kcal/mol}$$

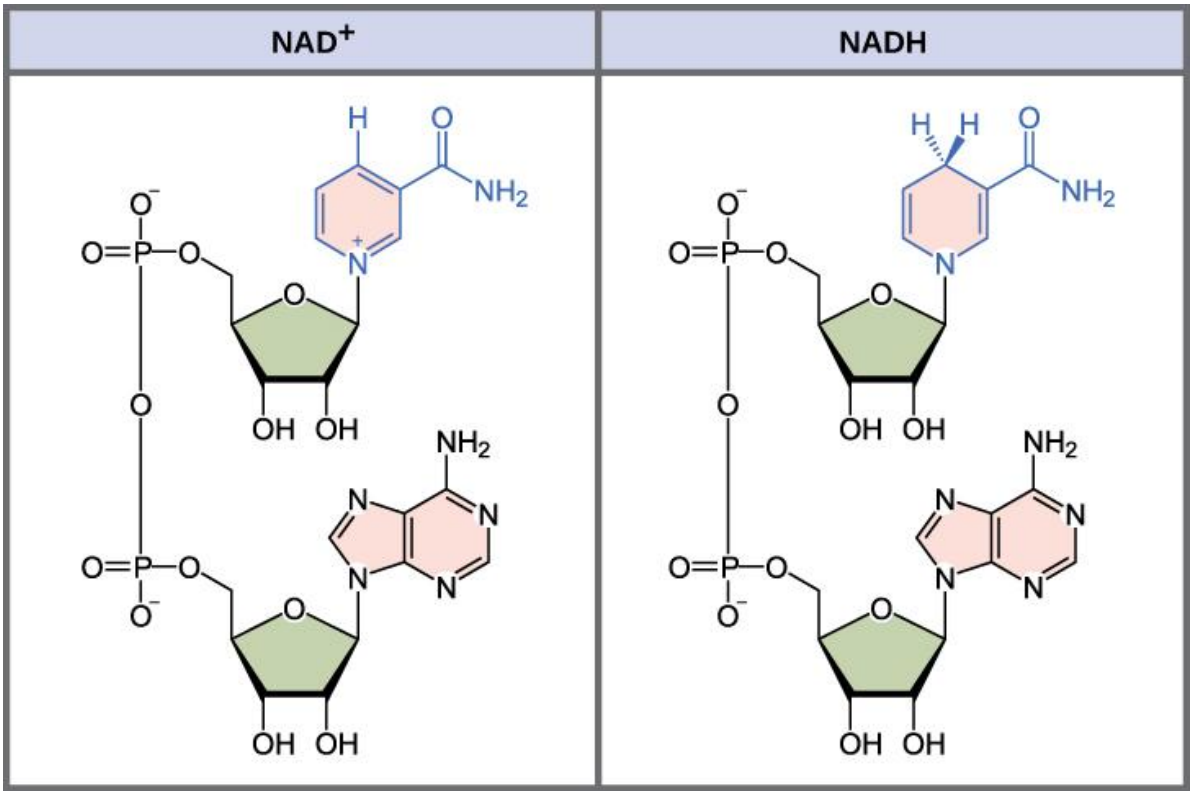
$$ATP + H_2O \rightarrow AMP + PP_i \quad \Delta G^\circ = -10.9 \text{ kcal/mol}$$

❖ Nicotinamide Adenine Dinucleotide (NAD)

Nicotinamide Adenine Dinucleotide (**NAD**) is a coenzyme found in all living cells. The compound is a dinucleotide, because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as **NAD^+** and **NADH** respectively.

In metabolism, nicotinamide adenine dinucleotide is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is, therefore, found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced. This reaction forms NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins, in posttranslational modifications. Because of the importance of these functions, the enzymes involved in NAD metabolism are targets for drug discovery.

Although NAD^+ is written with a superscript plus sign because of the formal charge on a particular nitrogen atom, at physiological pH for the most part it is actually a singly-charged anion (charge of minus 1), while NADH is a doubly-charged anion.



Nicotinamide adenine dinucleotide, like all *dinucleotide*s, consists of two nucleotides joined by a pair of bridging phosphate groups. The nucleotides consist of ribose rings, one with adenine attached to the first carbon atom (the 1' position) and the other with nicotinamide at this position. The nicotinamide moiety can be attached in two orientations to this anomeric carbon atom. Because of these two possible structures, the compound exists as two diastereomers. It is the β-nicotinamide diastereomer of NAD^+ that is found in organisms. These nucleotides are joined together by a bridge of two phosphate groups through the 5' carbons.

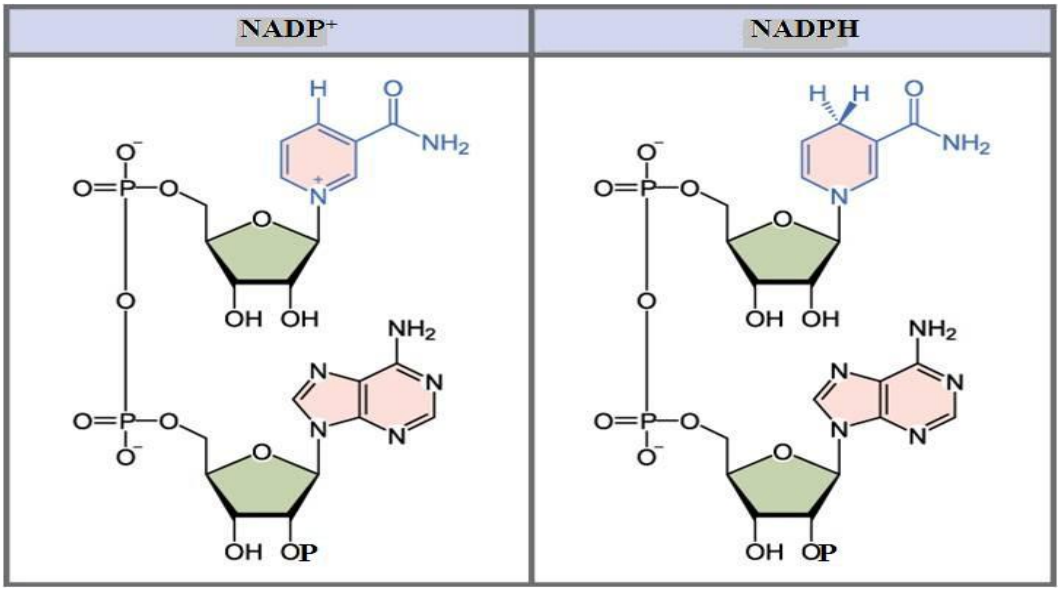
In metabolism, the compound accepts or donates electrons in redox reactions. Such reactions (summarized in formula below) involve the removal of two hydrogen atoms from the reactant (R), in the form of a hydride ion (H^-), and a proton (H^+). The proton is released into solution, while the reductant RH_2 is oxidized and NAD^+ reduced to NADH by transfer of the hydride to the nicotinamide ring.

$$RH_2 + NAD^+ \rightarrow NADH + H^+ + R;$$

From the hydride electron pair, one electron is transferred to the positively charged nitrogen of the nicotinamide ring of NAD^+, and the second hydrogen atom transferred to the C4 carbon atom opposite this nitrogen.

The reaction is easily reversible, when NADH reduces another molecule and is re-oxidized to NAD^+. This means the coenzyme can continuously cycle between the NAD^+ and NADH forms without being consumed.

- **Nicotinamide adenine dinucleotide phosphate (NADP)**



Nicotinamide adenine dinucleotide phosphate, abbreviated **$NADP^+$** or, in older notation, **TPN** (triphosphopyridine nucleotide), is a cofactor used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent.

NADPH is the reduced form of $NADP^+$. $NADP^+$ differs from NAD^+ in the presence of an additional phosphate group on the 2' position of the ribose ring that carries the adenine moiety.

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❖ Modes of energy yielding metabolism

ATP SYNTHESIS

The objective of a catabolic pathway is to make ATP that is to transform either chemical energy or electromagnetic (light) energy into the chemical energy contained within the high-energy bonds of ATP. Cells fundamentally can produce ATP in three ways:

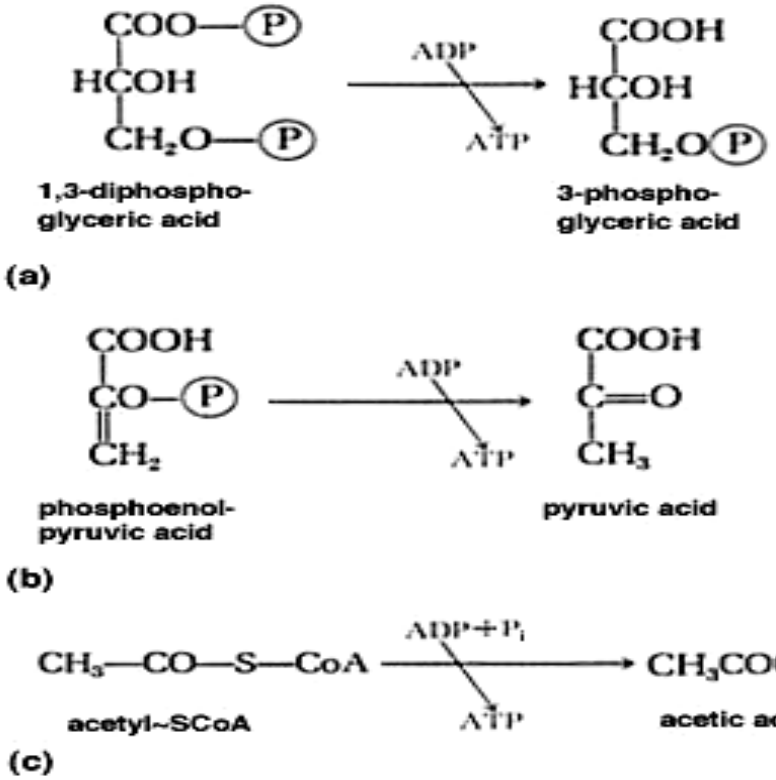
I) Substrate level phosphorylation
II) Electron transport phosphorylation (Oxidative Phosphorylation)
III) Photophosphorylation

I) Substrate level phosphorylation

Substrate-level phosphorylation is a type of metabolic reaction that results in the formation of adenosine triphosphate (ATP) or guanosine triphosphate (GTP) by the direct transfer and donation of a phosphoryl (PO_3) group to adenosine diphosphate (ADP) or guanosine diphosphate (GDP) from a phosphorylated reactive intermediate. Note that the phosphate group does not have to come directly from the substrate. By convention, the phosphoryl group that is transferred is referred to as a phosphate group.

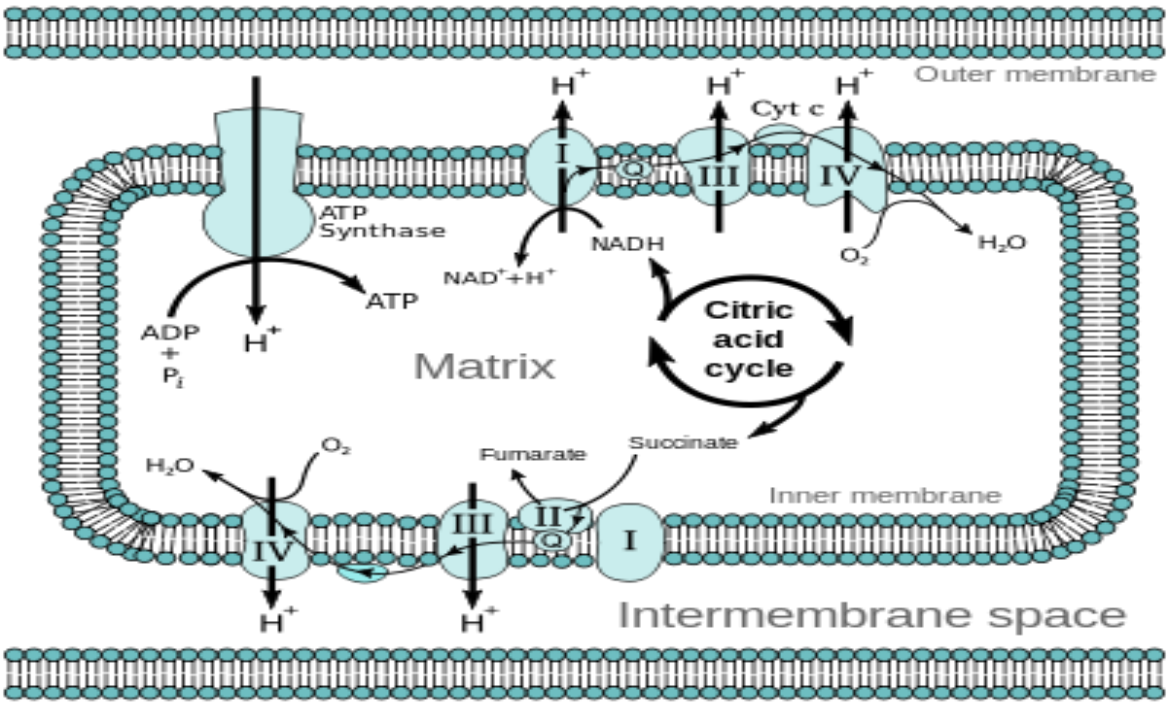
The main part of substrate-level phosphorylation occurs in the cytoplasm of cells as part of glycolysis and in mitochondria as part of the Krebs Cycle under both aerobic and anaerobic conditions. In the pay-off phase of glycolysis, two ATP are produced by substrate-level phosphorylation: two and only two 1,3-bisphosphoglycerate are converted to 3-phosphoglycerate by transferring a phosphate group to ADP by a kinase; two phosphoenolpyruvate are converted to pyruvate by the transfer of their phosphate groups to ADP by another kinase. The first reaction occurs after the generation of 1, 3-bisphosphoglycerate from 3-phosphoglyceraldehyde and an organic phosphate via a dehydrogenase. ATP is generated in a following separate step (key difference from oxidative phosphorylation) by transfer of the high-energy phosphate on 1, 3-bisphosphoglycerate to ADP via the enzyme phosphoglycerate kinase, generating 3-phosphoglycerate. As ATP is formed of a former inorganic phosphate group, this step leads to the energy yield of glycolysis. The second substrate-level phosphorylation occurs later by means of the reaction of phosphenolpyruvate (PEP) to pyruvate via the pyruvate kinase. This reaction

regenerates the ATP that has been used in the preparatory phase of glycolysis to activate glucose to glucose-6-phosphate and fructose-6-phosphate to fructose-1, 6-bisphosphate, respectively.



II) Electron transport phosphorylation (Oxidative Phosphorylation)

Oxidative phosphorylation (or OXPHOS in short) is the metabolic pathway in which the mitochondria in cells use their structure, enzymes, and energy released by the oxidation of nutrients to reform ATP. Although the many forms of life on earth use a range of different nutrients, ATP is the molecule that supplies energy to metabolism. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.



During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within the cell's intermembrane wall mitochondria, whereas, in prokaryotes, these proteins are located in the cells' intermembrane space. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *electron transport*. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase; this process is known as chemiosmosis. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

III) Photophosphorylation

In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called **photophosphorylation**. Only two sources of energy are available to living organisms: sunlight and reduction-

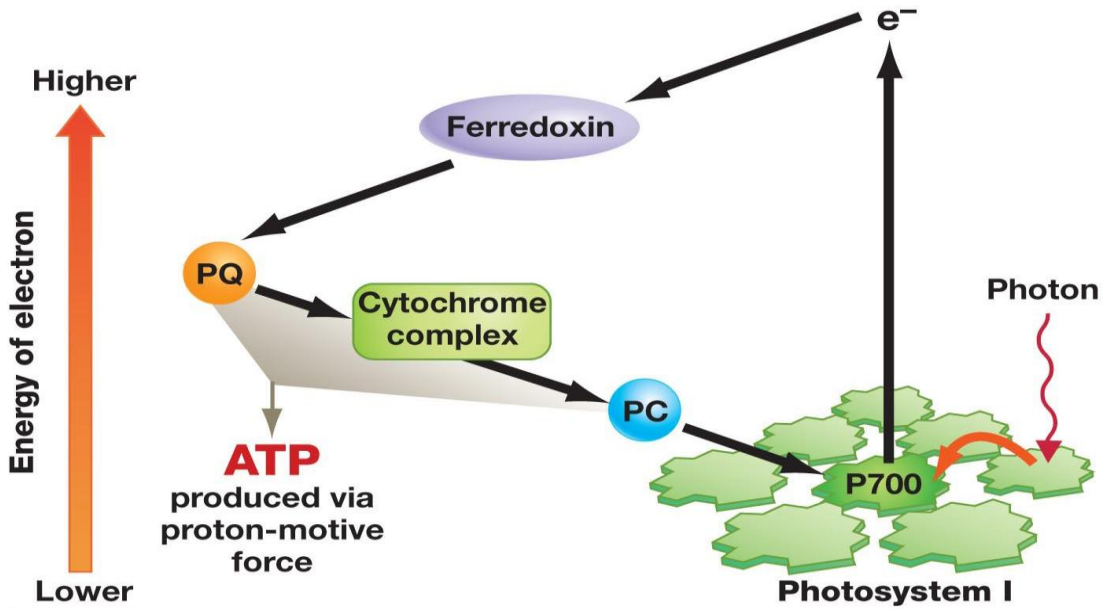
oxidation (redox) reactions. All organisms produce ATP, which is the universal energy currency of life.

In photophosphorylation, light energy is used to create a high-energy electron donor and a lower-energy electron acceptor. Electrons then move spontaneously from donor to acceptor through an electron transport chain.

Cyclic photophosphorylation

This form of photophosphorylation occurs on the thylakoid membrane. In cyclic electron flow, the electron begins in a pigment complex called photosystem I, passes from the primary acceptor to ferredoxin, then to cytochrome b_6f (a similar complex to that found in mitochondria), and then to plastocyanin before returning to chlorophyll. This transport chain produces a proton-motive force, pumping H^+ ions across the membrane; this produces a concentration gradient that can be used to power ATP synthase during chemiosmosis. This pathway is known as cyclic photophosphorylation, and it produces neither O_2 nor NADPH. Unlike non-cyclic photophosphorylation, NADP+ does not accept the electrons; they are instead sent back to cytochrome b_6f complex.

In bacterial photosynthesis, a single photosystem is used, and therefore is involved in cyclic photophosphorylation. It is favoured in anaerobic conditions and conditions of high irradiance and CO_2 compensation points.



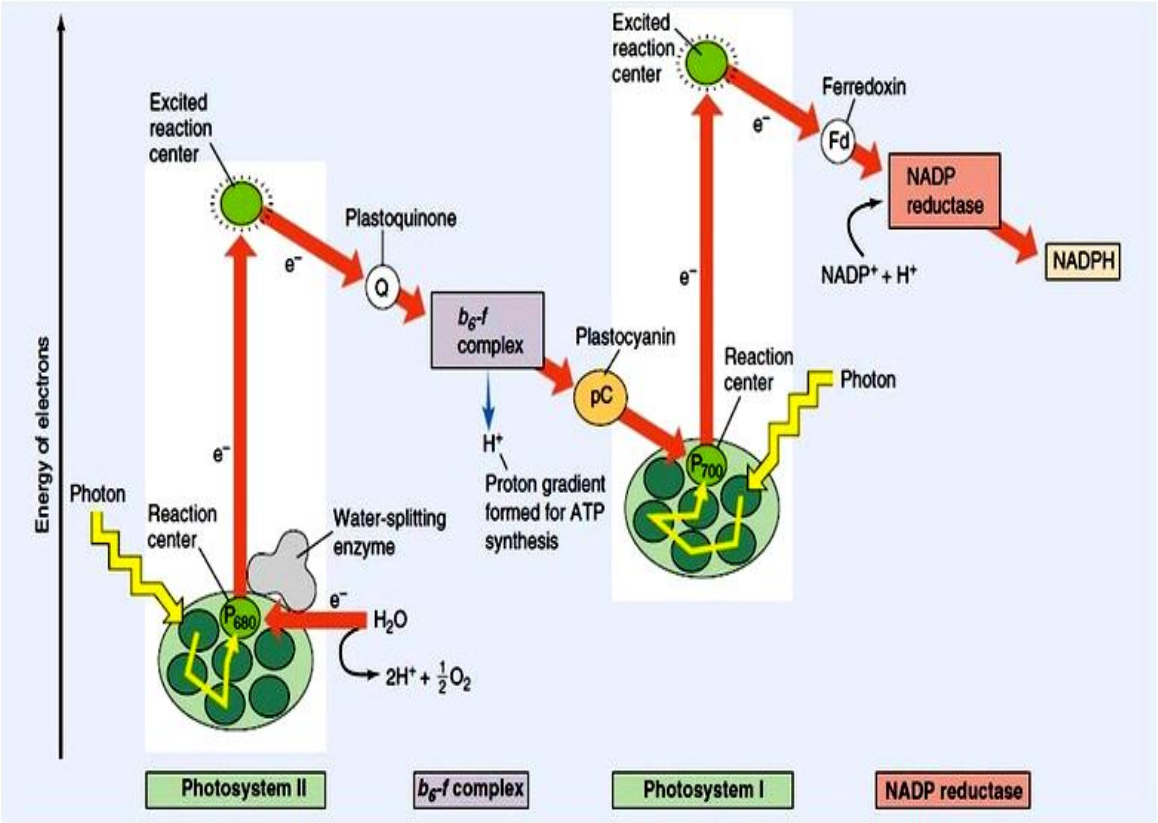
Non-cyclic photophosphorylation

The other pathway, non-cyclic photophosphorylation, is a two-stage process involving two different chlorophyll photosystems. Being a light reaction, non-cyclic photophosphorylation occurs in the frets or stroma lamellae. First, a water molecule is broken down into $2H^+ + 1/2\ O_2 + 2e^-$ by a process called photolysis (or *light-splitting*). The two electrons from the water molecule are kept in photosystem II, while the $2H^+$ and $1/2O_2$ are left out for further use. Then a photon is absorbed by chlorophyll pigments surrounding the reaction core center of the photosystem. The light excites the electrons of each pigment, causing a chain reaction that eventually transfers energy to the core of photosystem II, exciting the two electrons that are transferred to the primary electron acceptor, pheophytin. The deficit of electrons is replenished by taking electrons from another molecule of water. The electrons transfer from pheophytin to plastoquinone, which takes the $2e^-$ from Pheophytin, and two H^+ atoms from the stroma and forms PQH_2, which later is broken into PQ, the $2e^-$ is released to Cytochrome b_6f complex and the two H^+ ions are released into thylakoid lumen. The electrons then pass through the Cyt b_6 and Cyt f. Then they are passed to plastocyanin, providing the energy for hydrogen ions (H^+) to be pumped into the thylakoid space. This creates a gradient, making H^+ ions flow back into the stroma of the chloroplast, providing the energy for the regeneration of ATP.

The photosystem II complex replaced its lost electrons from an external source; however, the two other electrons are not returned to photosystem II as they would in the analogous cyclic pathway. Instead, the still-excited electrons are transferred to a photosystem I complex, which boosts their energy level to a higher level using a second solar photon. The highly excited electrons are transferred to the acceptor molecule, but this time are passed on to an enzyme called Ferredoxin-$NADP^+$ reductase which uses them to catalyse the reaction (as shown):

$$NADP^+ + 2H^+ + 2e^- \rightarrow NADPH + H^+$$

This consumes the H^+ ions produced by the splitting of water, leading to a net production of $1/2O_2$, ATP, and $NADPH+H^+$ with the consumption of solar photons and water.



The concentration of NADPH in the chloroplast may help regulate which pathway electrons take through the light reactions. When the chloroplast runs low on ATP for the Calvin cycle, NADPH will accumulate and the plant may shift from noncyclic to cyclic electron flow.

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❖ **Fermentation of carbohydrates**

There are 4 major pathways of carbohydrate breakdown in microorganisms. The sugars are broken down to pyruvate.

1) Glycolysis (EMP)
2) Hexose Monophosphate Pathway (HMP)
3) Entner Duodrof Pathway (ED)
4) Phosphoketolase Pathway

The EMP and HMP occur in both prokaryotes and eukaryotes. The ED and Phosphoketolase pathways occur only in prokaryotes. The organisms of

Enterobacteriaceae family mainly use EMP and HMP, while *Pseudomonadaceae* use ED pathway.

Fermentations are energy yielding pathways which utilize organic compounds as both electron donors and electron acceptors. Carbohydrates are the main substartes of fermentation.

1) Glycolysis / Embden-Mayerhof-Parnas Pathway (EMP pathway)

The sequence of reactions which converts glucose to pyruvic acid along with the production of ATP is known as Glycolysis. This pathway is also called as EMP pathway after the names of scientists Embden-Mayerhof-Parnas, who have discovered this pathway. It is found in both prokaryotes and eukaryotes.

Under anaerobic condition the pyruvate is converted into either lactic acid or ethanol and is called **Homolactic** or **Alcoholic** fermentation respectively. If the products are more than one such as Acetic acid, Ethanol, Propanol, Propionic acid etc along with lactic acid, then it is called **Heterolactic** fermentation. In aerobic organisms glycolysis leads to TCA cycle and electron transport chain, which releases most energy.

In glycolysis, each glucose molecule forms 2 pyruvate molecules. The breakdown takes place in a series of steps, each catalysed by specific enzyme. Most of the steps of glycolysis are reversible. All enzymes involved in glycolysis are present in cytoplasm i.e. glycolysis occurs in cytoplasm.

Glycolysis is divided into 2 phases—

A) Preparatory phase (Energy utilization phase)
B) Productive phase (Oxidative phase)

A) Preparatory phase (Energy utilization phase)

This has 4 steps

I) Phosphorylation of Glucose: -

Phosphorylation of glucose takes place and Glucose-6-phosphate is formed. The phospahate group is derived from ATP. The reaction is catalysed by *Hexokinase or Glucokinase*.

II) Isomerization of Glucose-6-phosphate to Fructose-6phosphate:-

Glucose-6-phosphate is isomerised to Fructose-6phosphate by *Phosphoglucoisomerase.*

III) Phosphorylation of Fructose-6-phosphate: -

Fructose-6-phosphate is phosphorylated to Fructose-1-6-diphosphate by an enzyme *Phsphofructokinase*. Here one more ATP is utilized.

IV) Cleavage of Fructose-1-6-diphosphate: -

Fructose-1-6-diphosphate splits into two 3 carbon compounds by *Aldolase.* The two compounds formed are Dihydroxy acetone phosphate (DHAP) and 3- Phosphoglyceraldehyde (3 PGAL).

DHAP can be converted into 3 PGAL by an enzyme *Triose phosphate isomerase.* DHAP cannot take part into further reactions of glycolysis. Only 3 PGAL take part into further reactions of glycolysis.

B) Productive phase (Oxidative phase)

This phase has 5 steps

I) Phsphorylation and oxidative dehydrogenation of 3 PGAL: -

This reaction is catalysed by *Glyceraldehyde phosphate dehydrogenase*. The coenzyme involved here is Nicotinamide Adenine Dinucleotide (NAD^+**).** When 3 PGAL is oxidized NAD^+ is reduced. The phosphorylation takes place on Carbon No. 1. The phosphoric acid (H_3PO_4) provides phosphate. 3 PGAL is converted into 1, 3 Diphosphoglycerate, a high-energy compound.

II) Formation of ATP from 1, 3 Diphosphoglycerate

The high energy phosphate group in 1, 3 Diphosphoglycerate is transfered to ADP resulting in the formation of 3 phosphoglycerate (3 PGA) and ATP by *phosphoglycerate kinase.*

III) Isomerization of 3 PGA into 2 PGA

The 3 Phosphoglyceric acid undergoes rearrangement to form 2 phosphoglyceric acid. The phopsphate group is transfered from 3^{rd} carbon to 2^{nd} carbon by an enzyme *Phosphoglyceromutase.*

IV) Dehydration of 2 PGA

The 2 PGA molecule is dehydrated by *Enolase* to form Phosphoenol pyruvate (PEP) which has a high energy enolic phosphate group.

V) Formation of ATP from PEP

The phosphate group from PEP is transffered to ADP to produce Pyruvic acid and ATP. The reaction is catalysed by *Pyruvate kinase.*

In anaerobic organisms, this pyruvic acid is reduced by NADH + H^+ which is formed in the V^{th} step and lactic acid is formed by *Lactate dehydrogenase.* Thus from one glucose molecule, two lactic acid molecules are formed. There is incomplete degradation of glucose in glycolysis.

In some organisms ethanol and carbon dioxide is produced by *Pyruvate dehydrogenase.*

In aerobic organisms, pyruvate enters into TCA cycle and oxidized completely to CO_2 & H_2O.

✶ Energetics of Glycolysis

2 ATP molecules are utilized in preparatory phase of glycolysis and from one 3 PGAL, two ATP molecules are produced and DHAP forms another 3 PGAL, which yields two more ATP molecules.

Thus in glycolysis two ATP molecules are utilized and 4 ATP molecules are produced i.e. there is net gain of only 2 ATP molecules in Glycolysis.

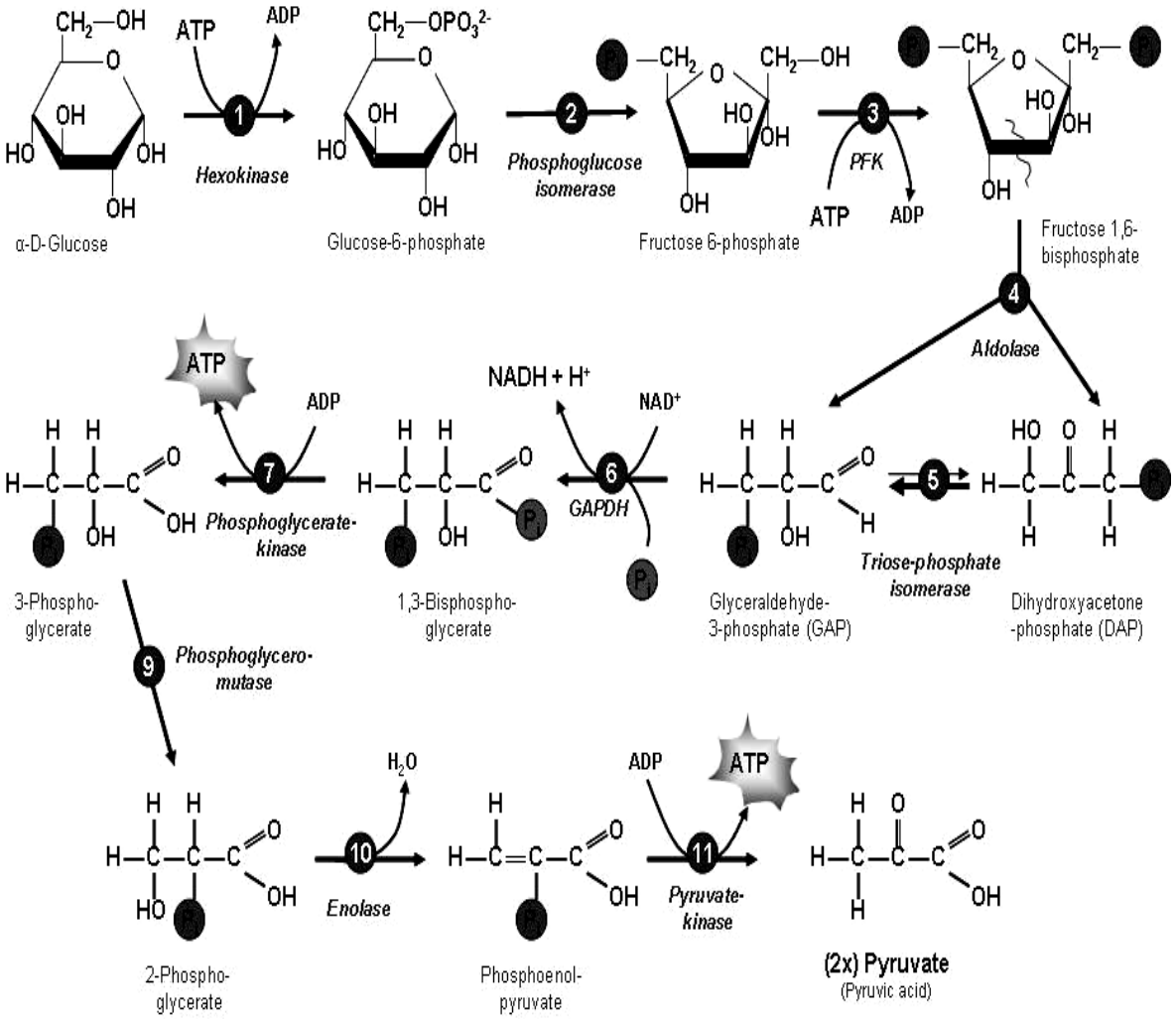
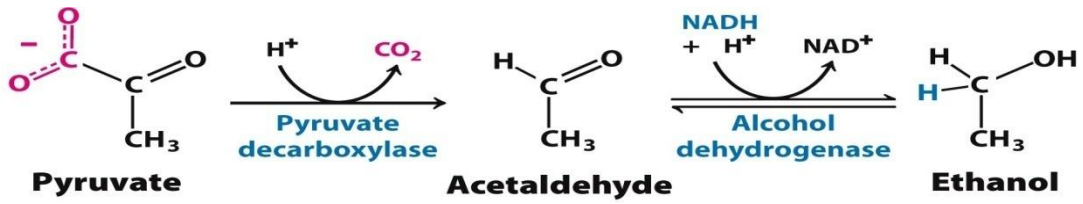


Fig. **Glycolytic pathway**

Alcoholic fermentation

Yeasts ferment glucose to ethanol and carbon dioxide. The fermentation pathway is identical to glycolysis except for terminal step catalysed by lactate dehydrogenase. In alcoholic fermentation, two pyruvate molecules are decarboxylated to acetaldehyde. Then acetaldehyde is reduced to ethanol with NADH + H^+ by enzyme ethanol dehydrogenase.



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2**. Hexose Monophosphate Pathway (HMP) /** Hexose monophosphate shunt (H.M.S.) / Pentose phosphate pathway (P.P.P.) / Phosphogluconate pathway (P.G.P.) / Warburg & Dickens's pathway (W&D P.)

Significance: -

1) Primary purpose of this pathway is to generate reducing power in terms of NADPH
2) Secondary function of this pathway is to convert hexoses to pentoses particularly ribose –5 phosphates are required for synthesis of nucleic acids.
3) The third function is oxidative degradation of pentose by conversing them to hexose, which then enters in the glycolysis.
4) This pathway is modified to participate in the formation of glucose from CO_2 in dark reactions of photosynthesis.

The HMP pathway functions in fermentation of several carbohydrates in many microorganisms & is a 'shunt' or 'loop' in the EMP. In this pathway one molecule of glucose-6–phosphate is converted into one molecule of glyceraldehyde, and three molecules of CO_2 are released. The HMP pathway yields only half the energy of the EMP pathway. It is not believed to be a major energy-yielding pathway in microorganism, rather it's function is to provide ribose phosphate for synthesis of nucleotides of RNA and to provide $NADPH_2$ as a source of reducing power.

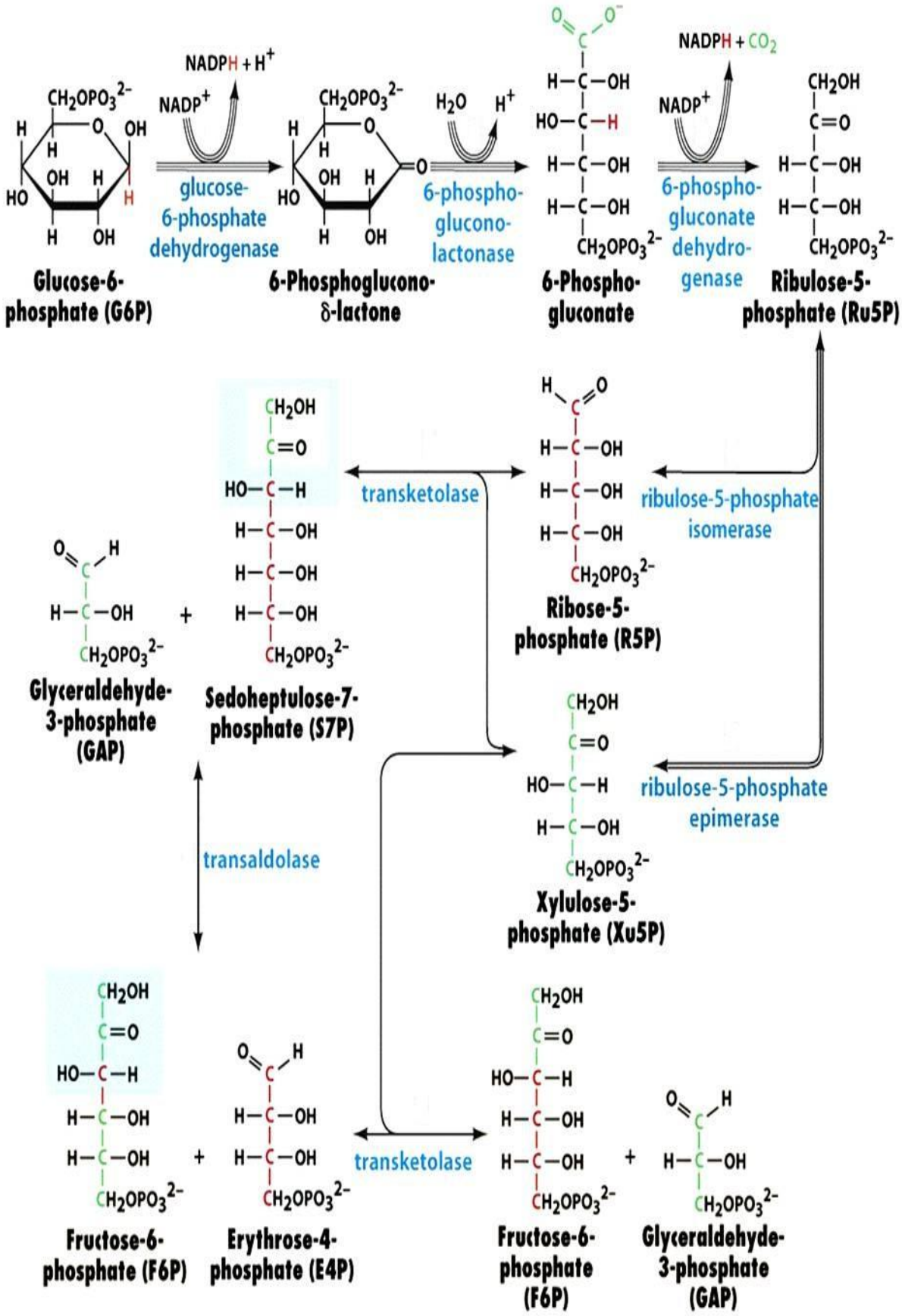
The reduction of NADP takes place at two stages,

1) Oxidation of glucose-6- phosphate to 6- phosphogluconate
2) Oxidation of 6- phosphogluconate to ribose –5- phosphate.

The reactions of HMP pathway are as follows: -

1) Glucose is phosphorylated by a phosphate group obtained from the breakdown of ATP to ADP to form *glucose-6-phpsphate. This* reaction is identical to that of the EMP pathway.
2) Glucose-6-phosphate is oxidized to the *δ*–lactone of phosphogluconic acid by an NADP-linked glucose –6-phosphate dehydrogenase.
3) The *d* lactone of phosphogluconic acid is immediately hydrolysed to 6-phosphogluconic acid by gluconolactonase. (D-glucono–*δ*–lactone hydrolase).

4) 6-phosphogluconic acid is simultaneously decarboxylated & oxidised to form D ribulose –5-phosphate. $NADP^+$ accepts the hydrogens & is reduced to NADPH + $H^{+\cdot}$ The reaction is catalysed by phosphogluconate dehydrogenase. The fourth reaction converts the hexose into a pentose.
5) Two different enzymes act upon the ribulose–5-phosphate. Ribulose phosphate-3-epimerase converts ribulose-5-phosphate to *xylulose–5-phosphate*, while ribose phosphate isomerase converts it to ribose–5-phosphate. Ribose–5-phosphate is a precursor for purine, pyrimidine & aromatic amino acid biosynthesis.
6) Xylulose–5-phosphate (C5) from *sedoheptulose*–7-phosphate (C7) & glyceraldehyde–3-phosphate (C3). Thus two pentose phosphate (C5) molecules react to give a heptose phosphate (C7) & a triose phosphate (C3). The reaction is catalysed by a *transketolase* in the presence of cofactors *thiamine pyrophosphate* (TTP) and Mg^{++}.
7) *Sedoheptulose phosphate* reacts with *glyceraldehyde-3- phosphate* to from *fructose–6- phosphate* and *erythrose-4- phosphate*. This *transaldolation reaction* is catalysed by *transaldolase*. The reaction is reversible. Erythrose – 4- phosphate is an important precursor for purine, pyrimidine and aromatic amino acid biosynthesis. Ribose-5- phosphate may also be the acceptor, in which case *octulose-8- phosphate* may be formed.
8) *Erythrose-4- phosphate* accepts a 2-carbon unit from *xylulose –5-phosphate* to form *fructose –6- phosphate* and *gleceraldehyde-6- phosphate*. This reaction is also catalysed by a *transketolase* as in reaction 6. The fructose *–6-phosphate* and the *glyceraldehyde-3- phosphate* of the pathway link up with the EMP pathway. *Fructose –6- phosphate* can be converted to *glucose-6-phosphate* with a *glucose phosphate isomerase (phosphoglucoisomerase)*. Glyceraldehyde-3- phosphate can also follow the reverse EMP pathway to form to *glucose-6- phosphate.*
9) The conversion of *fructose* 1, 6 *diphosphate* to fructose*–6-phosphate* requires an enzymes, hexose diphosphatase, because phosphofructokinase which catalyses reaction 3 in EMP pathway cannot do so in the reverse direction.
10) *Fructose –6- phosphate* gives rise to glucose-6-phosphate by the action of phosphoglucoisomerase, the enzyme which catalyses reaction 2 in the EMP pathway.


Glucose-6-phosphate (G6P)
NADPH + H+
NADP+
glucose-6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone
H2O
H+
6-phospho-glucono-lactonase
6-Phospho-gluconate
NADPH + CO2
NADP+
6-phospho-gluconate dehydro-genase
Ribulose-5-phosphate (Ru5P)
ribulose-5-phosphate isomerase
Ribose-5-phosphate (R5P)
transketolase
Glyceraldehyde-3-phosphate (GAP)
Sedoheptulose-7-phosphate (S7P)
ribulose-5-phosphate epimerase
Xylulose-5-phosphate (Xu5P)
transaldolase
transketolase
Fructose-6-phosphate (F6P)
Erythrose-4-phosphate (E4P)
Fructose-6-phosphate (F6P)
Glyceraldehyde-3-phosphate (GAP)

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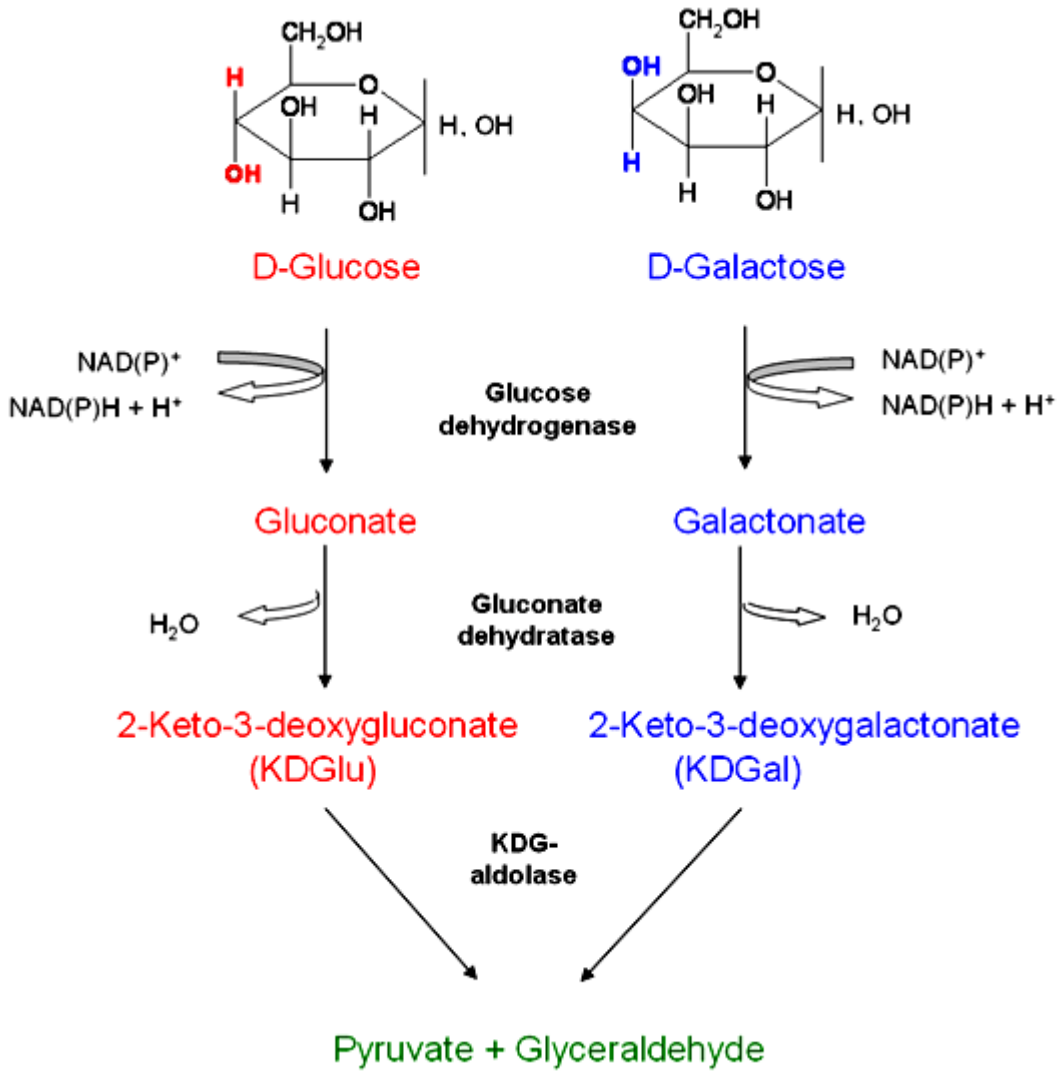
3) The Entner –Duodoroff (ED) pathway

This pathway was discovered by Entner and Duodoroff (1952) in the curse of metabolic studies on *Pseudomonas saccharophilia*, and has been since then fund in many their species of this genus. The first three reactions are identical to those of the HMP pathways. However, not certain whether the enzymes differ in their kinetic characteristics.

i. *Glucose* is phosphorylated to glucose–6-phosphate by ATP, the catalysing enzyme being hexokinase.
ii. Glucose-6 phosphate is oxidized with NADP-linked glucose-6 phosphate dehydrogenase to produce the δ-lactone of phosphogluconic acid (6-phosphoglucono-δ-lactone)
iii. 6-phosphoglucono-δ-lactone is immediately hydrolysed to 6-phosphogluconic acid by gluconolactanase (D-glucono- δ -lactone hydrolase).
iv. 6-phosphogluconic acid is hydrolysed by a phosphogluconate dehydratase to form a ketodeoxy sugar phosphate (2-keto-3-deoxy-6phosphogluconate: KDPG).
v. The ketodeoxy sugar phosphate is cleaved by an aldolase type enzyme (KDPG- aldolase) to pyruvate and glyceraldehyde-3 phosphate.This reaction is very similar to the cleavage of fructose 1, 6-diphosphate in the EMP pathway. KDPG catalyses the enolization of pyruvate.
vi. The metabolites of the ED pathway can lead to other glycolytic pathways:

a) Glucose-6 phosphate can follow the EMP pathway to yield fructose-6 phosphate ⟶ fructose-1,6-diphosphate ⟶dihydroxyacetone phosphate + glyceraldehyde -3 phosphate.
b) 6-phosphogluconic acid can lead to the HMP pathway.
c) Glyceraldehyde-3 phosphate is free to use the EMP pathway to pyruvate.
d) The importance of the pathway lies in the fact that the organisms can produce pentose precursors leading to purine and pyrimidine biosynthesis, as well as the biosynthesis of aromatic amino acids by a reverse HMP pathway. Glyceraldehyde can condense with fructose-6 phosphate to yield erythrose – 4 phosphate and xylulose- 5 phosphate. The reaction being catalysed by a transketolase. Xylulose- 5 phosphate is converted into ribulose- 5 phosphate by ribulose phosphate –3 epimerase. Erythrose-4 phosphate and fructose – 6 phosphate under the catalytic

action of an aldolase, form sedoheptulose- 7 phosphate and glyceraldehyde-3 phosphate. A second transketolase catalyses the formation of xylulose-5 phosphate and ribose –5 phosphate from the above two compounds.



The Entner –Duodoroff (ED) pathway

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(4) Phosphoketolase pathway

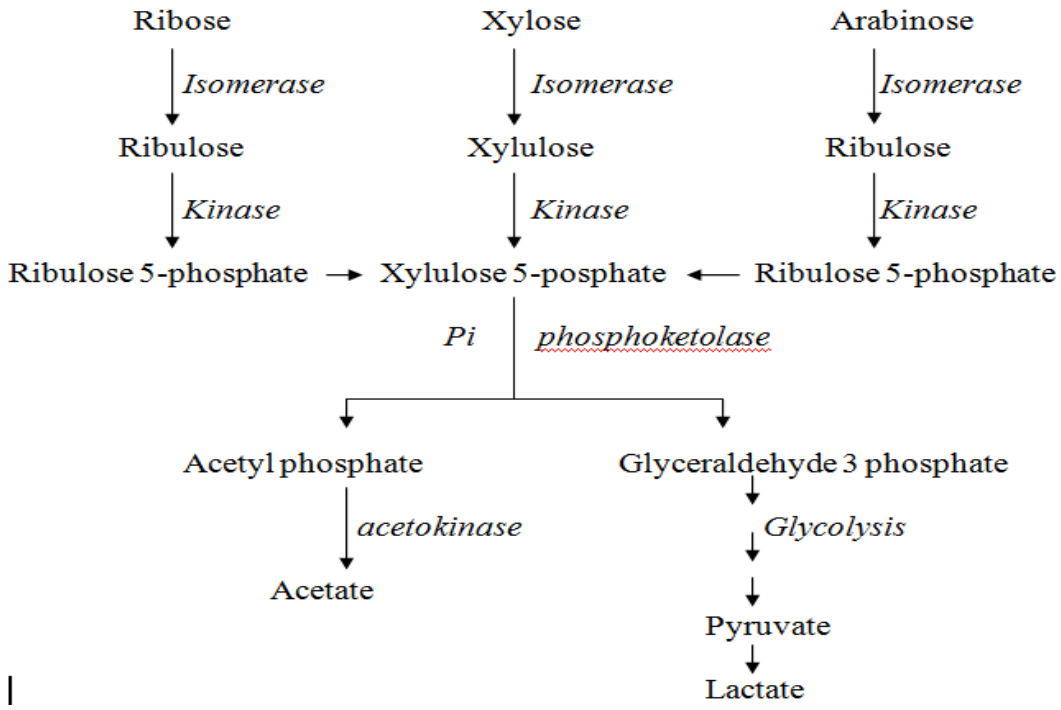
The heterofermentative *latobacilli* and the *bifidobacteria* possess the phosphoketolase pathway, which is a variation of the HMP pathway. There are two types of phosphoketolase pathway, the pentose phosphoketolase pathway and the hexose phosphoketolase pathway.

1. Pentose phosphoketolase pathway

The carbon source is ribose as well as other pentoses, Ribose-5 phosphate or xylulose-5 phosphate are formed via the HMP pathway. The pentose phosphoketolase pathway is found in *Leuconostoc mesenteroides* and *Leuconostoc plantarum*, in which the EMP, HMP and ED pathways are absent.

i. *Ribokinase* transfers a phosphate group from ATP to ribose, yielding *ribose-5-phosphate.*
ii. *Ribose-5-phosphate* is isomerised to *ribulose-5-phosphate* by ribose phosphate isomerase.
iii. *Ribulose phosphate-3-epimerase* converts ribulose-5-phosphate to *xylulose –5-phosphate.*
iv. All other pentoses are also converted to xylulos-5-phosphate. Xylose is isomerized to xylulose by *xylose isomerase.*
v. Xylulose is phosphprylated by ATP under the catalytic action of *xylulose kinase* to *xylulose-5-phosphate*.
vi. Arabinose is isomerised to ribulose by arabinose isomerase.
vii. Ribulose is phosphorylated by ATP to *ribulose-5-phosphate* by *ribulokinase.*
viii. *Ribulose-5-phosphate* is converted to *xylulose-5-phosphate* by *ribulosephosphate-4-epimerase.*
ix. Xylulose-5-phosphate plays a key role in the pentose phosphoketolase pathway, because the key enzyme of this pathway, phosphoketolase, reacts only with this compound. The phosphoketolase splits xylulose-5-phosphate into a *acetyl phosphate* and *glyceradehyde*-3-*phosphate*. The reaction requires *thiamine pyrophosphate* (TPP) and *inorganic phosphate* (Pi).
x. *Acetyl phosphate* is converted to *acetate* by *acetokinase.*
xi. *Glyceraldehyde-3-phosphate* is metabolised via the EMP pathway to *pyruvate* and finally *lactate.*

When glucose is the substrate, it is metabolised to ribulose-5-phosphate via the HMP pathway. Further metabolism is by the pentose phosphoketolase pathway.



2. Hexose Phosphoketolase pathways

This pathway is found in genus *bifidobacterium* (*Lactobacillus bifidus*), which lacks *glucose-6-phosphate dehydrogenase* and *fructose diphosphate aldolase*. Because of this, the EMP, HMP, ED or pentose phosphoketolase pathways cannot operate.

The key reaction is the cleavage of *fructose-6-phoshate* into *Erythrose-4-phosphate* and *acetyl phosphate* by *phosphokelase*.

In a reverse HMP pathway involving a *transealdolase* and a *transeketolase*, the compound *xylulose-5-phosphate* is formed. This is split into *glyceraldehyde-3-phosphate* and *acetyl phosphate*, as in the pentose phosphoketolase pathway.

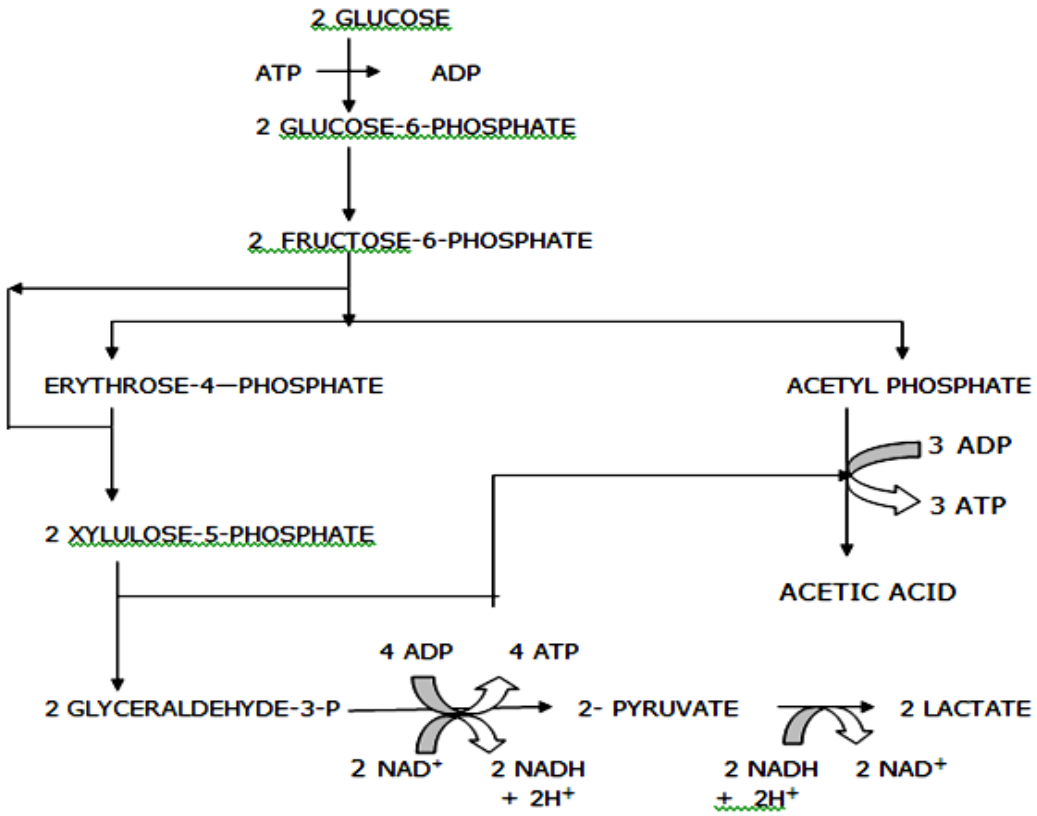


Fig. The hexose phosphoketolase pathway

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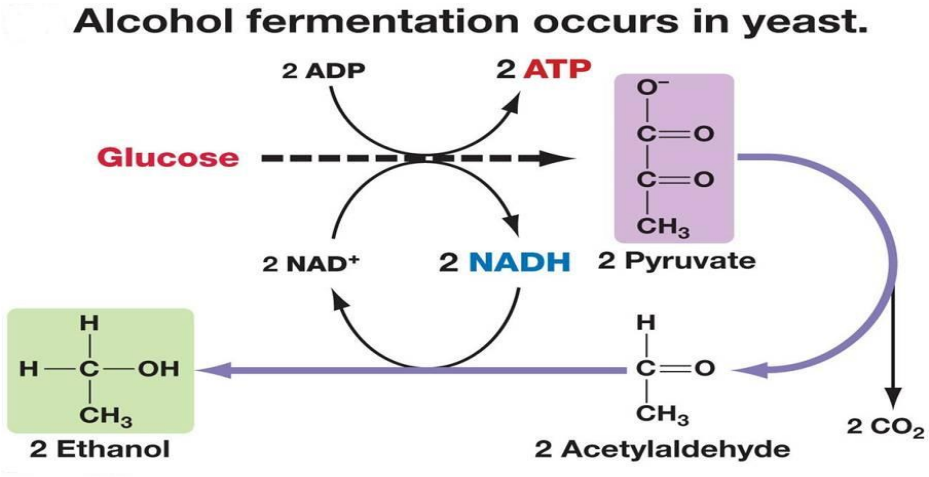
❖ **Alcoholic fermentation**

In alcoholic fermentation pyruvate is converted to *ethanol* and *carbon dioxide*. This process is characterise of yeasts, particularly strains of *Saccharomyces cerevisiae.* It is also found in some moulds and in the Mucorales, but is comparatively rare in bacteria. In the bacterium *Pseudomonas*, pyruvate is produced through the Entner-Duodoroff pathway. It is then metabolized to *ethanol* through *acetaldehyde.*

A molecule of *glucose* yields two molecules of pyruvate through the EMP pathway. Pyruvate metabolism takes place in two steps.

i. *Pyruvate* is first decarboxylated, yielding *acetaldehyde* and *carbon dioxide.* The reaction is catalysed through the enzyme *pyruvate decarboxylase,* with *thiamine pyrophosfhate* (TPP) as the coenzyme.

ii. *Acetaldehyde* is then reduced to *ethanol* by NADH+H+ ($NADH_2$) and NAD+ is regenerated. The catalysing enzyme is *alcohol dehydrogenase.*



It will be seen that the hydrogens removed during glucose metabolism are accepted by NAD, which is reduced to $NADH_2$. It is essential, that NAD be regenerated so that it can pick up more hydrogens. If this did not happen glycolysis would stop, resulting in the death of *he organism.

Regeneration of NAD takes place when *acetaldehyde* is reduced to *ethanol.* Alcoholic fermentation is of economic importance in the production of beverages and in raising bread.

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❖ **Lactic acid fermentation**

Lactic acid fermentation is a one-step reaction similar to glycolysis of mammalian cells. *Pyruvic acid* is reduced to *lactic acid,* the reaction being catalysed *by pyruvate reductase.* Lactic acid fermentation is characteristic of the lactic acid bacteria *(Lactobacillaceae)* which cause spoilage of food. Although morphologically heterogeneous, the bacteria are characterized by the fact that they produce *lactic acid* as the end product.

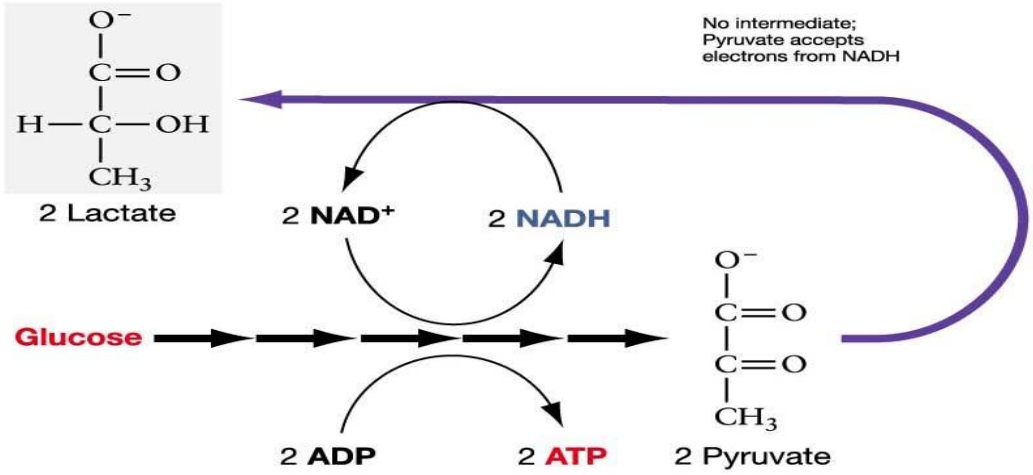
Glucose and other six-carbon sugars are converted into cellular energy and the metabolite lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells.

The *lactobacilli* are divided into two groups, *homofermentative* and *heterofermentative* strains. The demarcation between the two groups is indefinite in some cases.

a) Homolactic fermentation

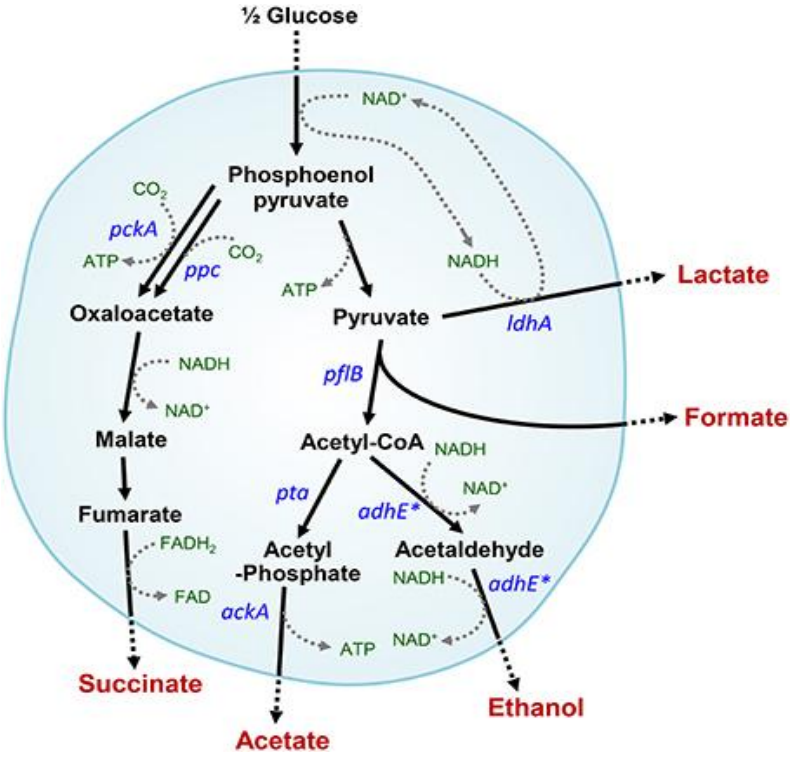
Homolactic fermentation is found in members of the genera *Strepto-coccus, Leuconostoc, Pediococcus* and *Lactobacillus.* In *homolactic fermentation,* one molecule of glucose is ultimately converted to two molecules of lactic acid.

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+.



b) Heterolactic fermentation (mixed acid fermentation)

Heterolactic fermentation is done by Lactobacillus and Leuconostoc. Early cleaving of the glucose molecule into ribulous 5 phosphate prevents glycolysis from happening in the first place. CO_2, ethanol, and lactate are all eventual products of heterolactic fermentation. Mixed-acid fermentation starts with glycolysis, then the pyruvate is reduced to Acetyl CoA which is reduced to acetate and ethanol. Other pyruvate is reduced to lactate and succinate in the presence of CO_2. CO_2 and H_2 are released from formate produced from the reduced pyruvate. Mainly the pathway of the *Enterobacteriaceae.* End products are a mixture of **lactic acid**, **acetic acid**, **formic acid**, **succinate** and **ethanol**, with the possibility of gas formation (**CO_2** and **H_2**) if the bacterium possesses the enzyme formate dehydrogenase, which cleaves formate to the gases.



❖ Butandiol fermentation

- Some *Erwinia, Klebsiella* and *Serratia* species produce 2, 3-butanediol in addition to lactate and ethanol from pyruvate, the EMP pathway product.
- Pyruvate is the substrate for one of three enzymes in these bacteria. These are lactate dehydrogenase, pyruvate:formate lyase and α-acetolactate synthase.
- The reactions catalyzed by these enzymes are similar to those of the mixed acid fermentation except for α-acetolactate synthase. This enzyme condenses two molecules of pyruvate to α-acetolactate that is further decarboxylated and reduced to 2, 3-butanediol.
- A similar metabolism is found in *Bacillus polymyxa* during vegetative growth and in lactic acid bacteria fermenting citrate.
- The first enzyme of this metabolism, α-acetolactate synthase, is best characterized in Gram-negative facultative bacteria. This enzyme has thiamine pyrophosphate as a cofactor to catalyze the following reactions:

$$CH_3–CO–COOH + E–TPP \longrightarrow CH_3–CHOH–TPP–E + CO_2$$

$$CH_3–CHOH–TPP–E + CH_3–CO–COOH \longrightarrow CH_3–C(=O)–CH3–COH–COOH + E\text{-}TPP$$

α-acetolactate

- Under anaerobic conditions, 2, 3-butanediol-producing facultative anaerobes produce acidic products, lowering the external and intracellular pH.
- α-acetolactate synthase, which catalyzes the first reaction to produce 2,3-butanediol, has an optimum at pH 6.0.
- When the intracellular pH drops, this enzyme becomes active to divert carbon flux from acid production to the neutral solvent.
- An enzyme catalyzing the same reaction catalyzes the first reaction of valine synthesis from pyruvate.
- This enzyme is referred to as the pH 8.0 enzyme while the enzyme involved in 2, 3-butanediol synthesis is referred to as the pH 6.0 enzyme.
- *Klebsiella pneumoniae, Klebsiella oxytoca* and *Enterobacter aerogenes* ferment glycerol to various products including 2, 3-butanediol.

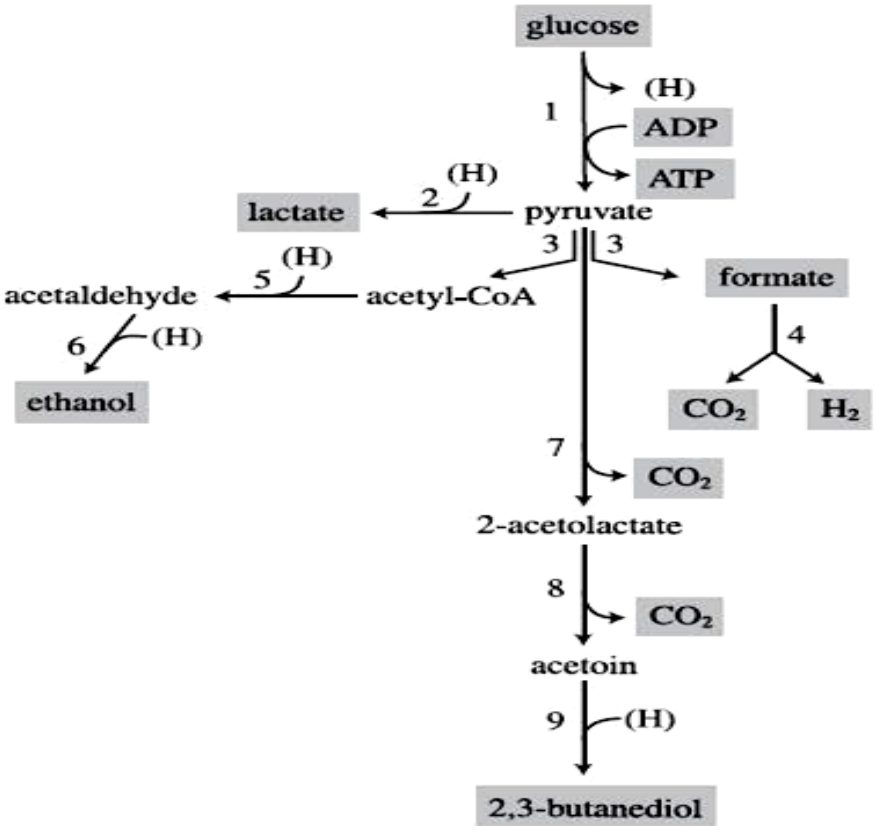


Fig. Butanediol fermentation by some Gram negative facultative anaerobic bacteria. Facultative anaerobes belong to the genera Erwinia, Klebsiella and Serratia and produce 2, 3-butanediol in addition to lactate and ethanol. 1, EMP pathway; 2, lactate dehydrogenase; 3, pyruvate:formate lyase; 4, formate:hydrogen lyase; 5, acetaldehyde dehydrogenase; 6, alcohol dehydrogenase; 7, α-acetolactate synthase; 8, α-acetolactate decarboxylase; 9, 2,3-butanediol dehydrogenase.

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❖ Butyric acid fermentation

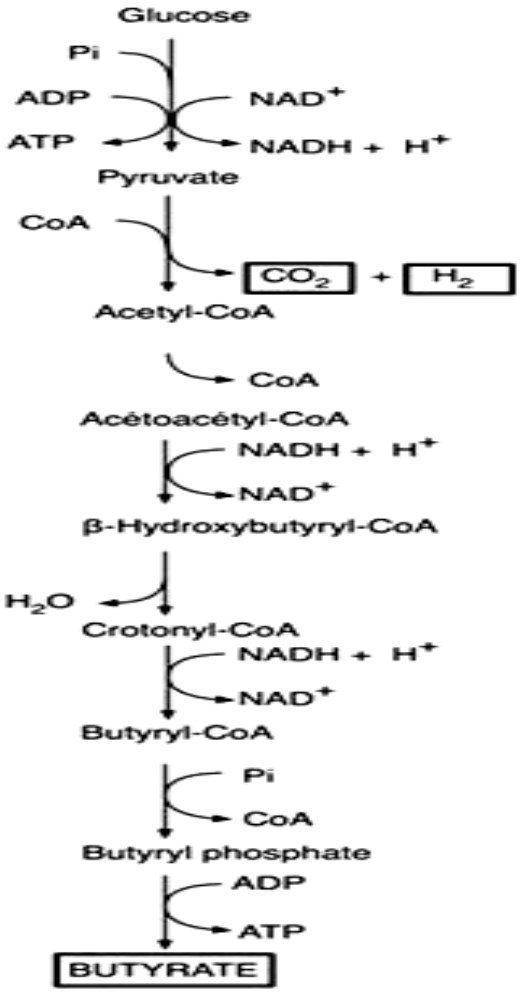
- Butyrate is produced as end-product of a fermentation process solely performed by obligate anaerobic bacteria.
- Examples of butyrate-producing species of bacteria: *Clostridium butyricum, Clostridium kluyveri, Clostridium pasteurianum, Fusobacterium nucleatum, Butyrivibrio fibrisolvens, Eubacterium limosum.*
- The pathway starts with the glycolytic cleavage of glucose to two molecules of pyruvate, as happens in most organisms. Pyruvate is then oxidized into acetyl coenzyme A using a unique mechanism that involves an enzyme system called pyruvate-ferredoxin oxidoreductase. Two molecules of carbon dioxide (CO_2) and two molecules of elemental hydrogen (H_2) are formed as waste products from the cell.
- Then, ATP is produced, as can be seen, in the last step of the fermentation. Three molecules of ATP are produced for each glucose molecule, a relatively high yield. The balanced equation for this fermentation is

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2\ CO_2 + 2\ H_2.$$

- Several species form acetone and *n*-butanol in an alternative pathway, which starts as butyrate fermentation. Some of these species are: *Clostridium acetobutylicum, Clostridium beijerinckii, Clostridium tetanomorphum, Clostridium aurantibutyricum*
- These bacteria begin with butyrate fermentation, as described above, but, when the pH drops below 5, they switch into butanol and acetone production to prevent further lowering of the pH. Two molecules of butanol are formed for each molecule of acetone.
- The change in the pathway occurs after acetoacetyl CoA formation. This intermediate then takes two possible pathways:

acetoacetyl CoA → acetoacetate → acetone

acetoacetyl CoA → butyryl CoA → butyraldehyde → butanol



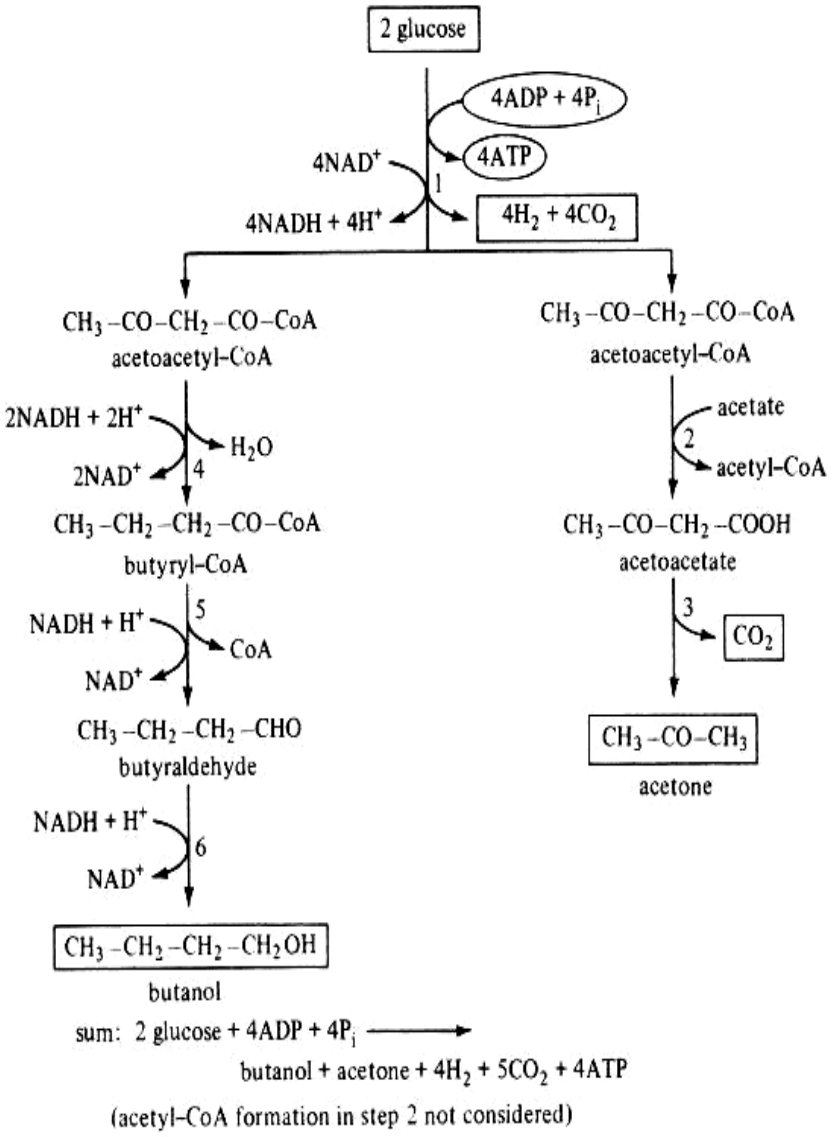
❖ Acetone Butanol fermentation

- A number of butyrate producing clostridia form small amounts of *n*-butanol.
- With a few species, however, a real shift from butyrate production to solvent production (*n*-butanol and acetone or isopropanol) can be observed under certain conditions.
- These species include *C. acetobutylicum, C. beijerinckii, C. tetanomorphum,* and *C. aurantibutyricum.*
- The most prominent species among these is *C. acetobutylicum,* which has been used on an industrial scale for the synthesis of *n*-butanol and acetone from molasses.
- During butanol fermentation the glycolytic reducing equivalents are reoxidized by reduction of butyryl-CoA to butanol via butyraldehyde. Therefore, 2 mol hexose have to be oxidized to gain the electrons required. The spare acetoacetyl-CoA is converted to acetoacetate and the

CoA is transferred to acetate, giving rise to acetyl-CoA and opening the opportunity for additional ATP synthesis in the acetate kinase reaction.

- Acetoacetate is decarboxylated to acetone, the second product of this fermentation. The overall reaction is:

$$2 \text{ glucose} \rightarrow \text{butanol} + \text{acetone} + 4H_2 + 5CO_2$$



Formation of acetone and butanol by C. acetobutylicum. 1, Reactions as in butyrate fermentation pathway (Phosphotransferase system and Embden-Meyerhof-Parnas pathway, pyruvate-ferredoxin oxidoreductase, hydrogenase, acetyl-CoA-acetyltransferase (thiolase)); 2, acetoacetyl-CoA: acetate coenzyme A transferase; 3, acetoacetate decarboxylase; 4, L(+)-,ß-hydroxybutyryl-CoA dehydrogenase, crotonase, and butyryl-CoA dehydrogenase; 5, butyraldehyde dehydrogenase; 6, butanol dehydrogenase.