Microbial metabolism Power Point No.1

- **Microbial metabolism**
- **Introduction**
- The term **metabolism** to refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy. In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism,** the breakdown of
- complex organic compounds into simpler ones. These reactions are called *catabolic,* or *degradative,* reactions.

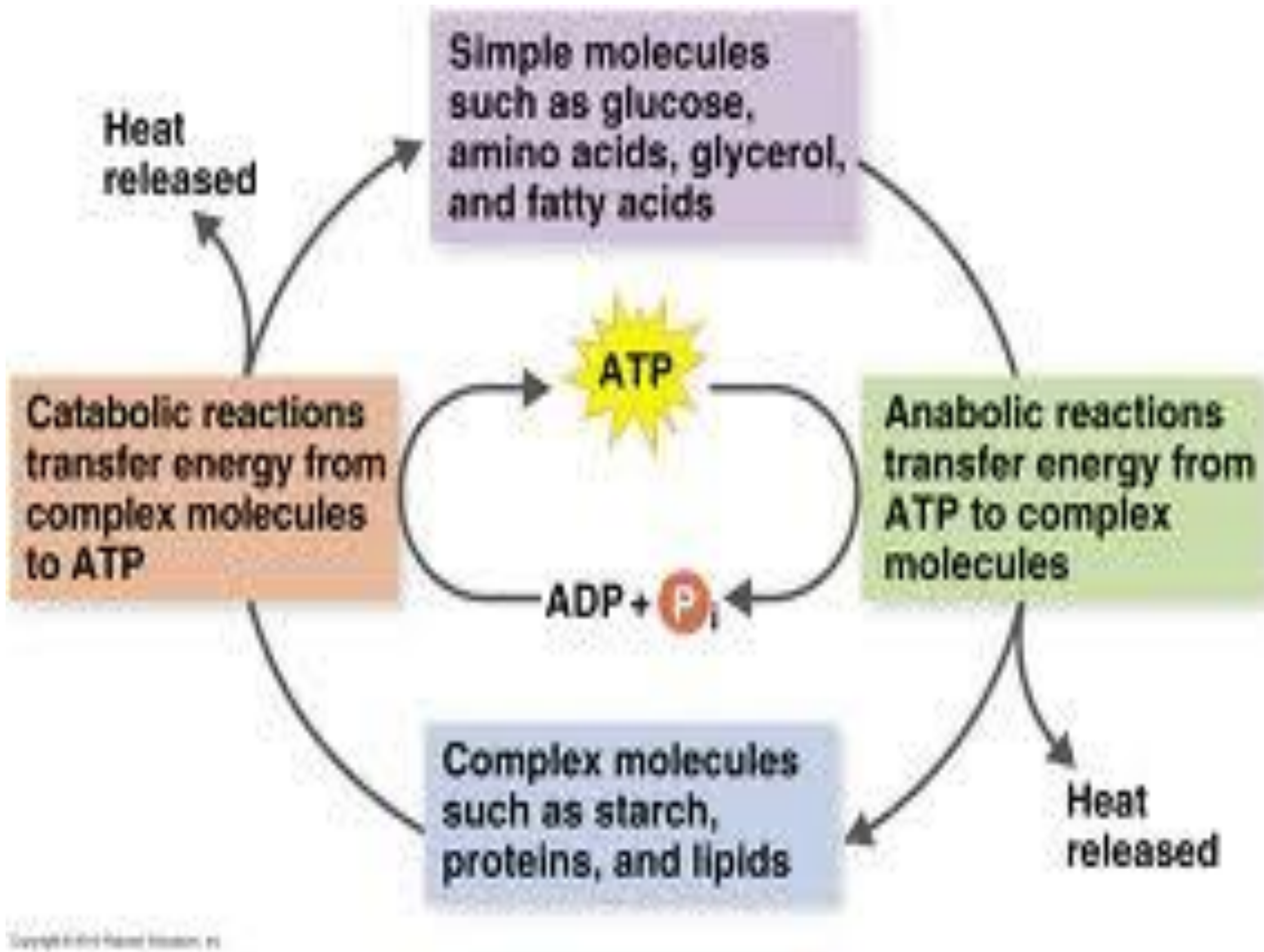
- Catabolic reactions are generally **hydrolytic reactions** (reactions which use water and in which chemical bonds are broken), and they are **exergonic** (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water. The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism,** the building of complex organic molecules from simpler ones. These reactions are called anabolic, or **biosynthetic reactions**. Anabolic processes often involve **dehydration synthesis** reactions (reactions that release water), and they are **endergonic** (consume more energy than they produce).

- Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions genera the materials for cell growth. Catabolic reactions provide building blocks for anabolic reactions and furnish the energy needed to drive anabolic reactions. This coupling of energy-requiring and energy-releasing reactions is made possible through the molecule adenosine-triphosphate (ATP). ATP stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work.

- One molecule of ATP consists of an adenine, a ribose, and three phosphate groups. When the terminal phosphate group is split from ATP, adenosine diphosphate (ADP) is formed, and energy is released to drive anabolic reactions. Using to represent a phosphate group (i, represents inorganic phosphate, which is not bound to any other molecule), we write this reaction as follows:
- $ATP \longrightarrow ADP + P_i + energy$
- Then, the energy from catabolic reactions is used to combine
- ADP and a p_i to resynthesize ATP:
- $ADP + P_i + energy \longrightarrow ATP$

- Thus, anabolic reactions are coupled to ATP breakdown, and catabolic reactions are coupled to ATP synthesis. This concept of coupled reactions is very important. For now, we should know that the chemical composition of a living cell is constantly changing: some molecules are broken down while others are being synthesized. This balanced flow of chemicals and energy maintains the life of a cell. The role of ATP in coupling anabolic and catabolic reactions is shown in figure below. Only part of the energy released in catabolism is actually available for cellular functions because part of the energy is lost to the environment as heat. Because the cell must use energy to maintain life, it has a continuous need for new external sources of energy.

The role of ATP in coupling anabolic and catabolic reactions



- A cell’s **metabolic pathways**(sequences of chemical reactions) are determined by its enzymes, which are in turn determined by the cell’s genetic make up. These enzymes are classified according to the function that catalyzed.
- **Oxidoreductase: Oxidation-reduction**, in which oxygen and hydrogen are gained or lost e.g. Cytochrome oxidase and lactate dehydrogenase
- **Transferase,** Transfer of functional groups, such as an amino group, acetyl group, or phosphate group eg. Acetate kinase and alanine deaminase
- **Hydrolase**, Hydrolysis (addition of water) eg. Lipase and glucosidase
- **Lyase,** Removal of groups of atoms. eg. Decarboxylase and isocitrate lyase

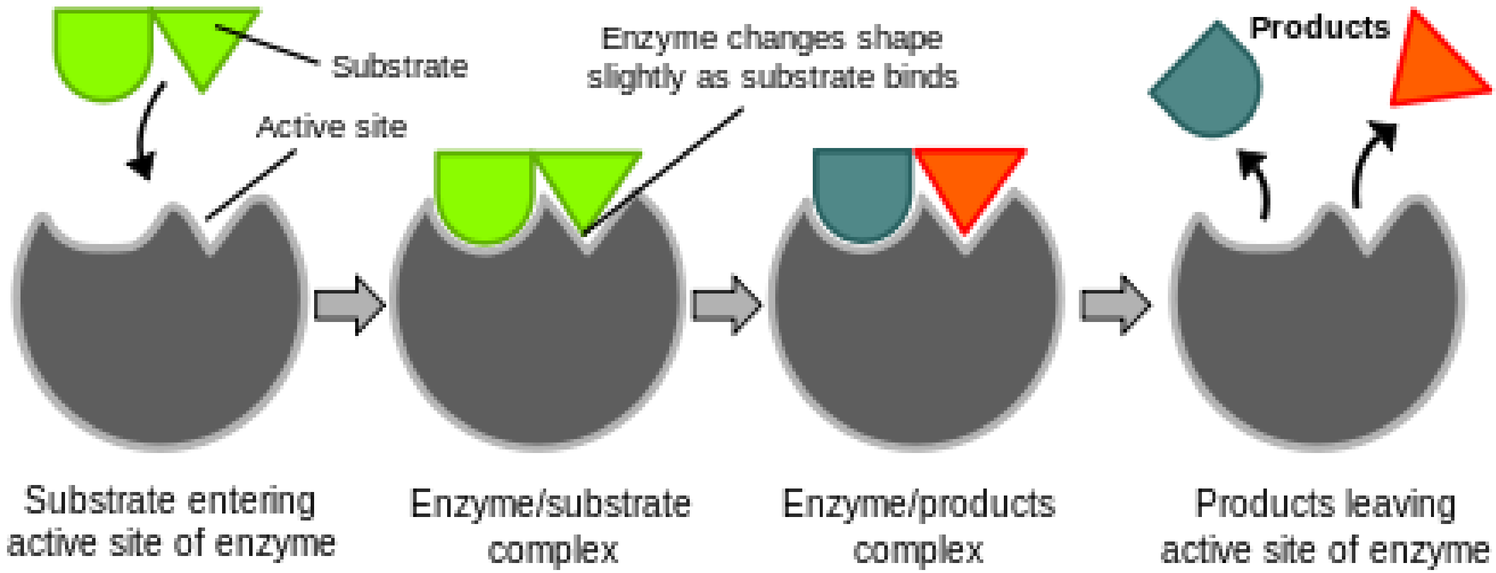
- **Isomerase,** Rearrangement of atoms within a molecule Glucose-phosphate isomerase and alanine racemase
- **Ligase,** Joining of two molecules (using energy usually derived from the breakdown of ATP) eg. Acetyl-CoA synthetase and DNA ligase .
- **Enzyme Components**
- Although some enzymes consist entirely of proteins, most consist of both a protein portion, called an **apoenzyme,** and a nonprotein component, called a **cofactor.** Ions of iron, zinc, magnesium, or calcium are examples of cofactors. If the cofactor is an organic molecule, it is called a **coenzyme,** such as vitamins**.** Apoenzymes are inactive by themselves; they must be activated by cofactors. Together, the apoenzyme and cofactor form a **holoenzyme,** or whole active enzyme . If the cofactor is removed, the apoenzyme will not function.

- **Selected Vitamin and Their Function**
- **Vitamin B1** (Thiamine): Part of coenzyme cocarboxylase; has many functions, including the metabolism of pyruvic acid.
- **Vitamin B2** (Riboflavin): Coenzyme in flavoproteins; active in electron transfers through respiratory system.
- **Niacin** (Nicotinic Acid): Part of NAD molecule; active in electron transfers through respiratory system.
- **Vitamin B6** (Pyridoxine) Coenzyme in amino acid metabolism.
- **Vitamin B12** (Cyanocobalamin); Coenzyme (methyl cyanocobalamide) involved in the transfer of methyl groups; active in amino acid metabolism.

- **Pantothenic Acid:** Part of coenzyme A molecule; involved in the metabolism of pyruvic acid and lipids
- **Biotin:** Involved in carbon dioxide fixation reactions and fatty acid synthesis
- **Folic Acid:** Coenzyme used in the synthesis of purines and pyrimidines
- **Vitamin E:** Needed for cellular and macromolecular syntheses
- **Vitamin K:** Coenzyme used in electron transport (naphthoquinones and quinones), instead of coenzyme Q.

- **The Mechanism of Enzymatic Action**
- Enzymes lower the activation energy of chemical reactions. The general sequence of events in enzyme action is as follows:
- 1-The surface of the substrate contacts a specific region of the surface of the enzyme molecule, called the **active site.**
- 2- A temporary intermediate compound forms, called an **enzyme–substrate complex.**
- 3-The substrate molecule is transformed by the rearrangement of existing atoms, the breakdown of the substrate molecule, or in combination with another substrate molecule.
- 4-The transformed substrate molecules(the products of the reaction) are released from the enzyme molecule because they no longer fit in the active site of the enzyme.

- 5-The unchanged enzyme is now free to react with other substrate molecules.
- As a result of these events, an enzyme speeds up a chemical reaction.
- **Substrate + Enzyme** $\longrightarrow$ **Es-complex** $\longrightarrow$ **product + E**



- **Carbohydrate Metabolism and Energy Production**
- In the study of carbohydrate metabolism there are several major reactions to be considered.
- 1- The pathway used for the degradation of carbohydrates.
- 2- The mechanisms used for the production of biologically useful energy in the form ATP, that is, whether ATP is
- generated via **a**-substrate level phosphorylation, or **b**- oxidative phosphrylation.

- 3- The metabolic steps in which reducing activity is generated and used **a**-to reduce pyruvate or other substrates to form end products, or **b**- for biosynthetic reactions requiring reducing action.
- 4- production of intermediates that serve as precursors of amino acid, purine and Pyrmidines and other vital cellular constitutes.
- 5- The effect of oxygen on the metabolic and energy-generating reactions that is, whether these reactions are primarily **a**- aerobic, **b**- anaerobic , and **c**- facultative.
- It should be understood that carbohydrates are not the only common compound utilized as a source of energy production by microorganisms. A wide variety of other compounds , including fatty acids, lipids, and amino acids can also be utilized by certain microorganisms for the production of energy as well as for biosynthesis.

Glycolysis

- **T**he oxidation of glucose to pyruvic acid, is usually the first stage in carbohydrate catabolism. Most microorganisms use this pathway which is occurs in most living cells. Glycolysis is also called the *Embden-Meyerhof pathway.* The word *glycolysis* means splitting of sugar, and this is exactly what happens. The enzymes of glycolysis catalyze the splitting of glucose, a six-carbon sugar, into two three-carbon sugars. These sugars are then oxidized, releasing energy, and their atoms are rearranged to form two molecules of pyruvic acid .During glycolysis NAD^+ is reduced to NADH, and there is a net production of two ATP molecules by substrate-level phosphorylation. .

- Glycolysis does not require oxygen; it can occur whether oxygen is present or not. This pathway is a series of ten chemical reactions, each catalyzed by a different enzyme. To summarize the process, glycolysis consists of two basic stages, a preparatory stage and an energy-conserving stage:
- 1. First, in the preparatory stage (steps 1 – 4, in Figure below), two molecules of ATP are used as a six-carbon glucose molecule is phosphorylated, restructured, and split into two three-carbon compounds: glyceraldehyde 3-phosphate (GP) and dihydroxyacetone phosphate (DHAP). Step 4 DHAP is readily converted to GP. The conversion of DHAP into GP means that from this point on in glycolysis, two molecules of GP are fed into the remaining chemical reactions.

- 2. In the energy-conserving stage (steps 6-10 , in Figure below), the two three-carbon molecules are oxidized in several steps to two molecules of pyruvic acid. In these reactions, two molecules of NAD^+ are reduced to NADH, and four molecules of ATP are formed by substrate-level phosphorylation. Because two molecules of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, there is a net gain of two molecules of ATP for each molecule of glucose that is oxidized. In yeast, pyruvate is cleaved by pyruvate carboxylase to form acetaldehyde and carbon dioxide and then acetaldehyde is reduced to ethanol by alcohol dehydrogenase. In lactic acid bacteria, pyruvate is reduced to lactate by lactate dehydrogenase.

Final step of glycolysis in yeasts and bacteria

- $CH_3COCOOH \longrightarrow CH_3CHO + CO_2$ In yeast
- $CH_3CHO + NADH + H^+ \longrightarrow 2CH_2OH + NAD^+$
- in yeast

- $CH_3COCOOH + NADH + H \longrightarrow CH_3CHOHCOOH + NAD^+$
- in bacteria
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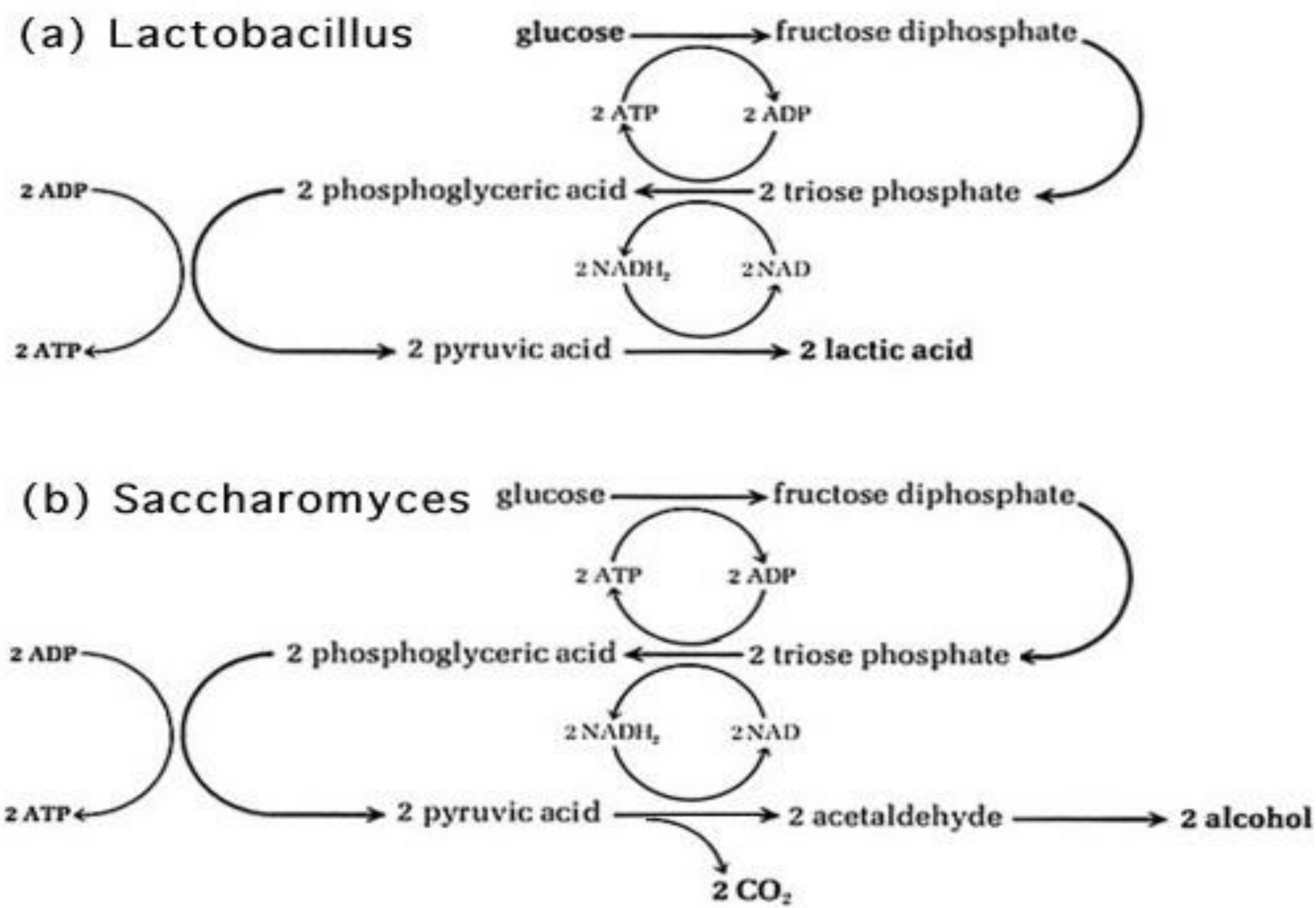
The Embden Meyerhof pathway of lactic acid fermentation in lactic acid bacteria (*Lactobacillus*) and pathway of alcohol fermentation in yeast (*Saccharomyces*).



- **10 Steps in glycolysis**
- **Step 1:** The enzyme hexokinase phosphorylates (adds a phosphate group to) glucose in the cell's cytoplasm. In the process, a phosphate group from ATP is transferred to glucose producing glucose 6-phosphate. Glucose ($C_6H_{12}O_6$) + hexokinase + ATP → ADP + Glucose 6-phosphate ($C_6H_{11}O_6P_1$).
- **Step 2:** The enzyme phosphoglucoisomerase converts glucose 6-phosphate into its isomer fructose 6-phosphate. Isomers have the same molecular formula, but the atoms of each molecule are arranged differently.
  Glucose 6-phosphate ($C_6H_{11}O_6P_1$) + Phosphoglucoisomerase → Fructose 6-phosphate ($C_6H_{11}O_6P_1$)

- **Step 3**
  The enzyme phosphofructokinase uses another ATP molecule to transfer a phosphate group to fructose 6-phosphate to form fructose 1, 6-diphosphate.
  Fructose 6-phosphate ($C_6H_{11}O_6P_1$) + phosphofructokinase + ATP → ADP + Fructose 1, 6-diphosphate ($C_6H_{10}O_6P_2$)
  Step 4
  The enzyme aldolase splits fructose 1, 6-diphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate and glyceraldehyde phosphate.
  Fructose 1, 6-diphosphate ($C_6H_{10}O_6P_2$) + aldolase → Dihydroxyacetone phosphate ($C_3H_5O_3P_1$) + Glyceraldehyde phosphate

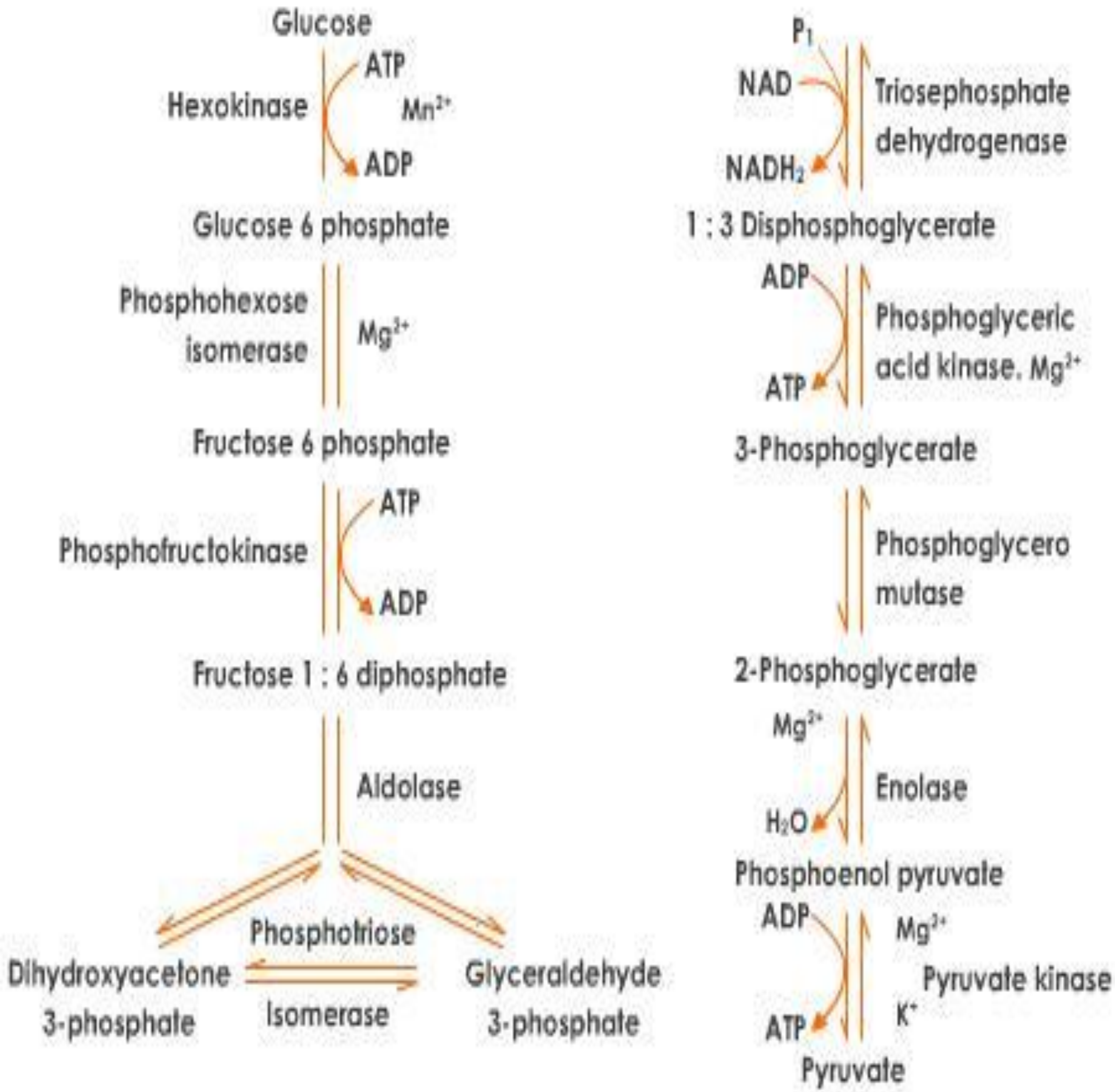
- **Step 5:** The enzyme triose phosphate isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate and glyceraldehyde phosphate. Glyceraldehyde phosphate is removed as soon as it is formed to be used in the next step of glycolysis. Dihydroxyacetone phosphate ($C_3H_5O_3P_1$) $\rightarrow$ Glyceraldehyde phosphate ($C_3H_5O_3P_1$) Net result for steps 4 and 5: Fructose 1, 6-diphosphate ($C_6H_{10}O_6P_2$) $\leftrightarrow$ 2 molecules of Glyceraldehyde phosphate ($C_3H_5O_3P_1$)

- **Step 6:**The enzyme triose phosphate dehydrogenase serves two functions in this step. First the enzyme transfers a hydrogen (H^-) from glyceraldehyde phosphate to the oxidizing agent nicotinamide adenine dinucleotide (NAD^+) to form NADH. Next triose phosphate dehydrogenase adds a phosphate (P) from the cytosol to the oxidized glyceraldehyde phosphate to form 1, 3-diphoshoglyceric acid. This occurs for both molecules of glyceraldehyde phosphate produced in step 5.
  A. Triose phosphate dehydrogenase + 2 H^- + 2 NAD^+ → 2 NADH + 2 H^+
  B. Triose phosphate dehydrogenase + 2 P + 2 glyceraldehyde phosphate ($C_3H_5O_3P_1$) → 2 molecules of 1,3-diphoshoglyceric acid ($C_3H_4O_4P_2$)

- **Step 7:**The enzyme phosphoglycerokinase transfers a P from 1,3-diphoshoglyceric acid to a molecule of ADP to form ATP. This happens for each molecule of 1,3-diphoshoglyceric acid. The process yields two 3-phosphoglyceric acid molecules and two ATP molecules.
  2 molecules of 1,3-diphoshoglyceric acid ($C_3H_4O_4P_2$) + phosphoglycerokinase + 2 ADP → 2 molecules of 3-phosphoglyceric acid ($C_3H_5O_4P_1$) + 2 ATP

- **Step 8:** The enzyme phosphoglyceromutase relocates the P from 3-phosphoglyceric acid from the third carbon to the second carbon to form 2-phosphoglyceric acid. 2 molecules of 3-Phosphoglyceric acid ($C_3H_5O_4P_1$) + phosphoglyceromutase → 2 molecules of 2-Phosphoglyceric acid ($C_3H_5O_4P_1$)
  Step 9: The enzyme enolase removes a molecule of water from 2-phosphoglyceric acid to form phosphoenolpyruvic acid (PEP). This happens for each molecule of 2-phosphoglyceric acid.
  2 molecules of 2-Phosphoglyceric acid ($C_3H_5O_4P_1$) + enolase → 2 molecules of phosphoenolpyruvic acid (PEP) ($C_3H_3O_3P_1$)

- **Step 10:**The enzyme pyruvate kinase transfers a P from PEP to ADP to form pyruvic acid and ATP. This happens for each molecule of PEP. This reaction yields 2 molecules of pyruvic acid and 2 ATP molecules.
2 molecules of PEP ($C_3H_3O_3P_1$) + pyruvate kinase + 2 ADP → 2 molecules of pyruvic acid ($C_3H_4O_3$) + 2 ATP
- **Summary**: In summary, a single glucose molecule in glycolysis produces a total of 2 molecules of pyruvic acid, 2 molecules of ATP, 2 molecules of NADH and 2 molecules of water. Although 2 ATP molecules are used in steps 1-3, 2 ATP molecules are generated in step 7 and 2 more in step 10. This gives a total of 4 ATP molecules produced. If you subtract the 2 ATP molecules used in steps 1-3 from the 4 generated at the end of step 10, you end up with a net total of 2 ATP molecules produced.



Glucose
ATP
Hexokinase
Mn2+
ADP
Glucose 6 phosphate
Phosphohexose
isomerase
Mg2+
Fructose 6 phosphate
ATP
Phosphofructokinase
ADP
Fructose 1 : 6 diphosphate
Aldolase
Phosphotriose
Dihydroxyacetone
3-phosphate
Isomerase
Glyceraldehyde
3-phosphate
P1
NAD
Triosephosphate
dehydrogenase
NADH2
1 : 3 Disphosphoglycerate
ADP
Phosphoglyceric
acid kinase. Mg2+
ATP
3-Phosphoglycerate
Phosphoglycero
mutase
2-Phosphoglycerate
Mg2+
Enolase
H2O
Phosphoenol pyruvate
ADP
Mg2+
Pyruvate kinase
K+
ATP
Pyruvate

- **Functions of Glycolysis**
- a) It is only in this process glucose is converted into pyruvic acid.
- b) The intermediate products formed in glycolysis are utilized for the synthesis of fat and amino acids.
- c) It also yields some energy in the form of ATP.

1. **Homolactic Fermentation**:

- **Lactic acid** is the sole end product. Pathway of the homolactic acid bacteria (*Lactobacillus* and most streptococci). The bacteria are used to ferment milk and milk products in the manufacture of yogurt, buttermilk, sour cream, cottage cheese, cheddar cheese, and most fermented dairy products.

2. **Mixed Acid Fermentations:**

- Mainly the pathway of the E*nterobacteriaceae.*

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.

- 2a. **Butanediol Fermentation**. Forms mixed acids and gases as above, but, in addition, **2,3 butanediol** from the condensation of 2 pyruvate. The use of the pathway decreases acid formation (butanediol is neutral) and causes the formation of a distinctive intermediate, **acetoin**. (butanediol formers), such as *Klebsiella* and *Enterobacter*.
- 3. **Butyric acid fermentations**, as well as the butanol-acetone fermentation (below), are run by the Clostridia, the masters of fermentation. In addition to butyric acid, the clostridia form acetic acid, CO_2 and H_2 from the fermentation of sugars. Small amounts of ethanol and isopropanol may also be formed.

- 3a. **Butanol-acetone fermentation**. Butanol and acetone were discovered as the main end products of fermentation by *Clostridium acetobutylicum* during the World War I. This discovery solved a critical problem of explosives manufacture (acetone is required in the manufacture gunpowder).
- 4. **Propionic acid fermentation**. This is an unusual fermentation carried out by the propionic acid bacteria which include *Corynebacteria*, *Propionibacterium* and

Bifidobacterium.

- Although sugars can be fermented straight through to propionate, propionic acid bacteria will ferment lactate (the end product of lactic acid fermentation) to acetic acid, CO_2 and propionic acid. The formation of propionate is a complex and indirect process involving 5 or 6 reactions. Overall, 3 moles of lactate are converted to 2 moles of propionate + 1 mole of acetate + 1mole of CO_2, and 1 mole of ATP.