

## Chapter 8 Microbial Metabolism

- Overview of metabolism
- Enzymes
- Glucose catabolism
  - Glycolysis
  - Fermentation
  - Respiration

# Cell Metabolism Overview

## Carbon and energy sources

Ex.

- Carbohydrates
- Amino acids, Proteins
- Lipids

**Uptake**

### Catabolism

- Energy (ATP)
- Carbon skeletons
- Reducing power (Electrons)

### Anabolism

- Cell walls
- Membranes
- Enzymes
- Nucleic acids

**Excretion**

## Waste products

- Ex.
- CO<sub>2</sub>
  - Organic acids (Ex. acetic, lactic)
  - Ethanol
  - Nitrogenous wastes

## A. Overview of metabolism

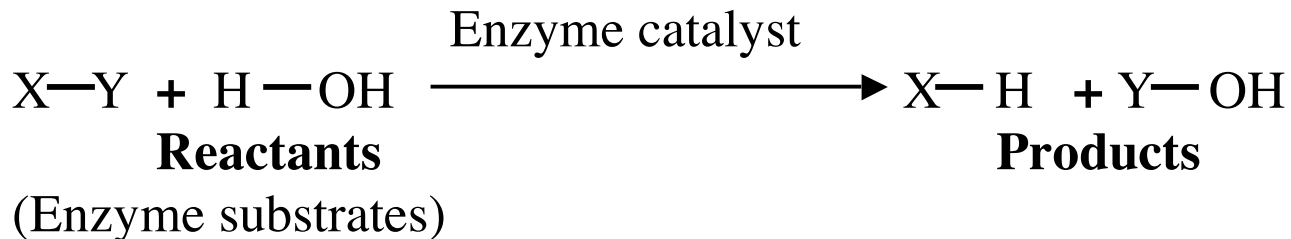
**Metabolism** The chemical reactions in a cell that support life and reproduction

- Metabolic reactions can be divided into two types
  1. **Anabolism** Reactions involved in biosynthesis of cellular components
    - Ex. Proteins, nucleic acids, lipids, carbohydrates
    - Needed for growth, reproduction and repair of cell
    - Reactions require energy, organic building blocks and inorganic nutrients
  2. **Catabolism** Reactions that break down organic molecules
    - Ex. Oxidation of glucose
    - Produce energy and building blocks for:
      - anabolic reactions
      - cell movement
      - transport of substance across membranes

## B. Enzymes

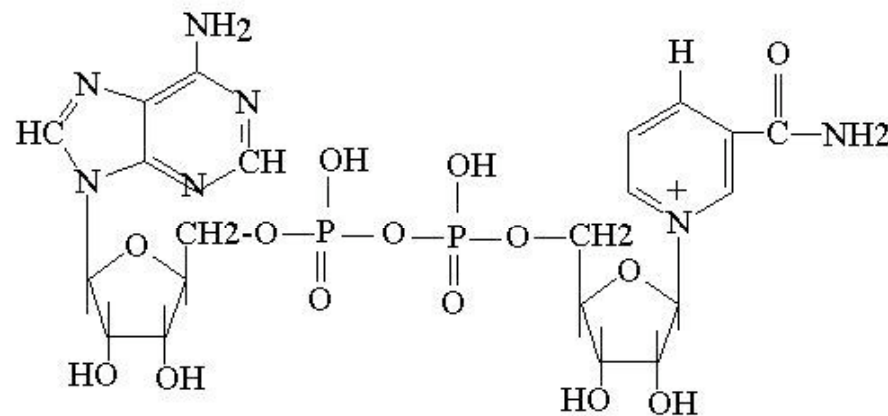
- Cells must perform thousands of different metabolic reactions.
- Chemical reactions are too slow to sustain life processes.
- **Enzymes** are catalysts that speed up metabolic reactions in which chemical bonds are broken and formed
  - Proteins composed of ~100's to 1000's of amino acids
- Each metabolic reaction has its own enzyme
  - Each enzyme is specific for:
    - 1.) **Type of chemical reaction** it catalyzes
    - 2.) **Reactants** (substrates) and products of the reaction

**Ex.**



➤ Enzymes are not changed by the reaction and catalyze the same reaction over and over

- **Active site** Enzyme location where substrates bind and react and where products are formed and released
  - The active site is like a lock and the enzyme's substrate is the only key that fits the lock
- Some enzymes are composed only of amino acids, others contain additional **cofactors** that aid catalysis
  - Some cofactors are **inorganic ions**
    - Ex.  $\text{Fe}^{++}$
  - **Organic cofactors** are called **coenzymes**
    - Ex.  $\text{NAD}^+$  (Nicotinamide adenine dinucleotide)



## Table 8.2

Figure 8.2

Figure 8.4

## Figure 8.5 Carrier functions of coenzymes

## Figure 8.7 Constitutive and regulated enzymes

Figure 8.10

## Enzyme names

- Add “-ase” suffix to substrate or type of reaction

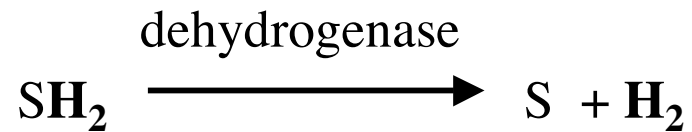
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**Ex.**

- **Lipase** Catalyzes reaction that converts lipids into glycerol and fatty acids



- **Dehydrogenase** H is removed from the substrate



# Main Classes of Metabolic Reactions

## 1. **Condensation** or **ligation** --joining reactions

- Synthesize complex molecules through formation of covalent bonds between smaller molecules
- A **H<sub>2</sub>O** molecule is often **removed** during covalent bond formation

## 2. **Hydrolysis** --breaking apart with water

- Breakdown complex organic molecules
- A **H<sub>2</sub>O** molecule is **added** to break a covalent bond

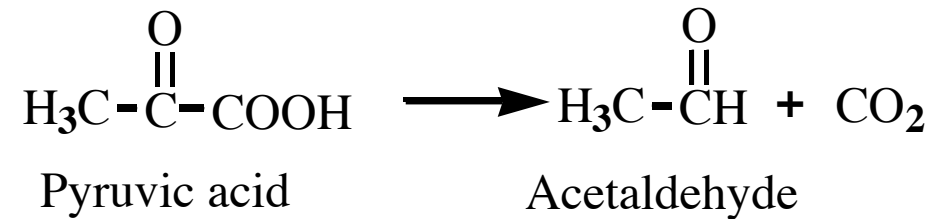
### 3. Group transfer --addition or removal of functional groups

**Ex.**

1.) Addition of a phosphate group



2.) Removal of a carboxyl group

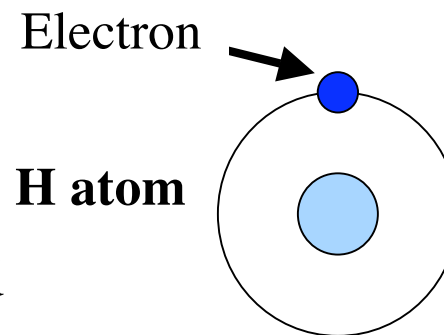
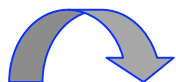


#### 4. Oxidation-reduction reactions

**Oxidation** = removal of electrons (or H)

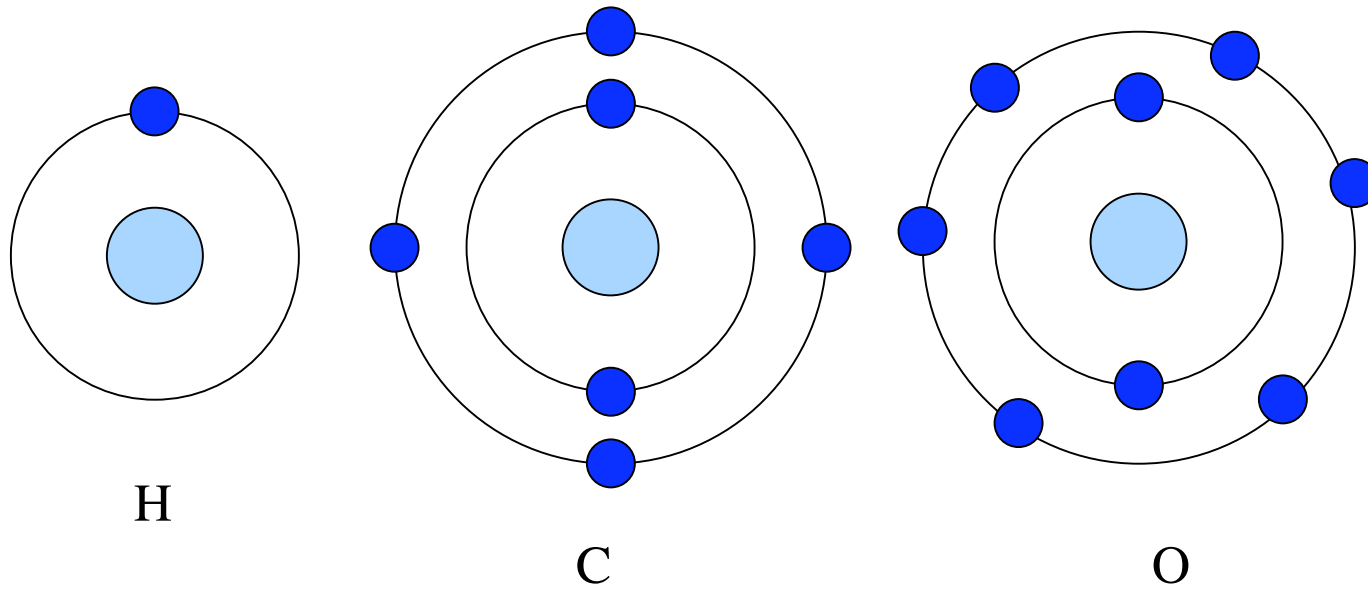
**Reduction** = addition of electrons (or H)

-Transfers 2 electrons or (2 H atoms) from one compound to another



- Valence electrons

Electrons in outer shell of an atom



A hydrogen atom has 1 valence electron

A carbon atom has 4 valence electrons

An oxygen atom has 6 valence electrons

- Atoms are neutral because the number electrons = number of protons
- Oxidation number describes the positive or negative character of an atom or ion that has gained or lost valence electrons

## Oxidation-Reduction (Redox) Reactions

**Oxidation** = removal of electrons (or H)

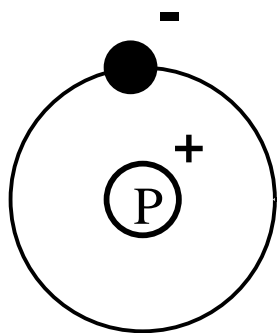
**Reduction** = addition of electrons (or H)

- Electrons are removed from one molecule or ion (oxidation) and transferred to another (reduction)
- Electrons may be transferred:

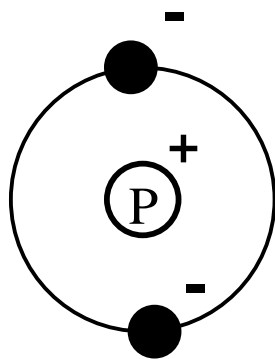
1. as a single electron

2. with a H atom

3. in pairs with a hydride anion



**H** atom carries  
1 electron

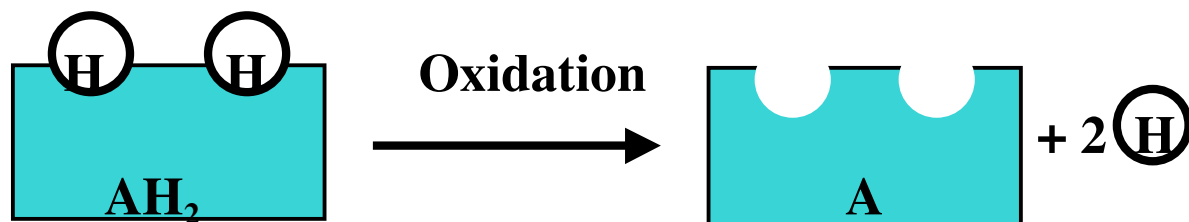


Hydride anion, **H<sup>-</sup>**, carries  
2 electrons

- Oxidation-reduction reactions involve two half reactions that occur together



### 1. Oxidation half reaction



### 2. Reduction half reaction

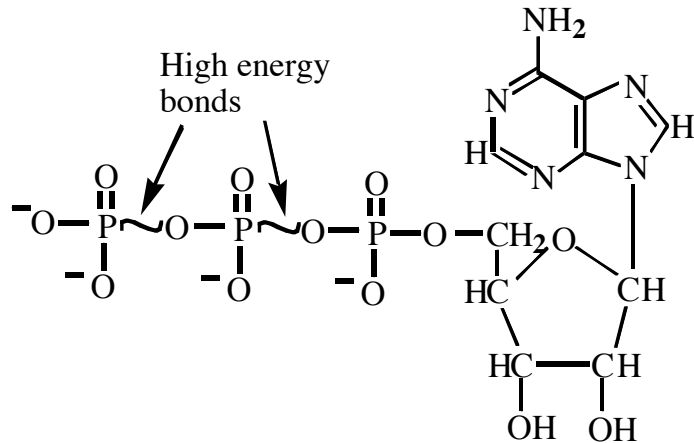


- A was oxidized by removal of 2 electrons (as 2 H atoms) while B was reduced by addition of 2 electrons (also as 2 H atoms)



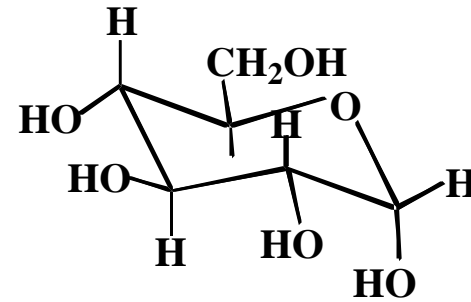
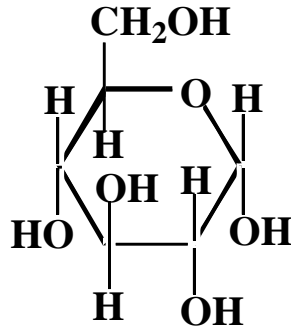
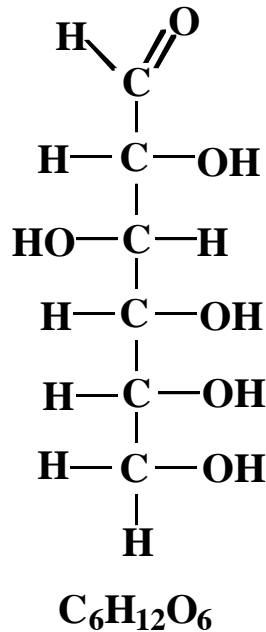
➤ **Oxidation-reduction reactions release energy**

- Some of the energy can be captured and stored in high energy covalent bonds between the phosphate groups of adenosine triphosphate (ATP)



- The captured energy can be used by the cell to do work
  - Form new bonds during synthesis of macromolecule
  - Transport of substances across the cell membrane
  - Motility

# Glucose Catabolism



- Contains usable energy stored in covalent bonds between atoms
- Nutrient used by many organisms as:
  - Source of energy for synthesis of ATP
  - Carbon and Hydrogen for synthesis of cell constituents
- Presence or absence of O<sub>2</sub> during catabolism determines:
  - Amount of energy available to the cell
  - Type of metabolic end products

# Overview of Glucose Catabolism

## 1. Glycolysis

- Glucose is oxidized to pyruvic acid,  $\text{NAD}^+$  is reduced to NADH and 2 **ATPs** are produced

## 2. Fermentation

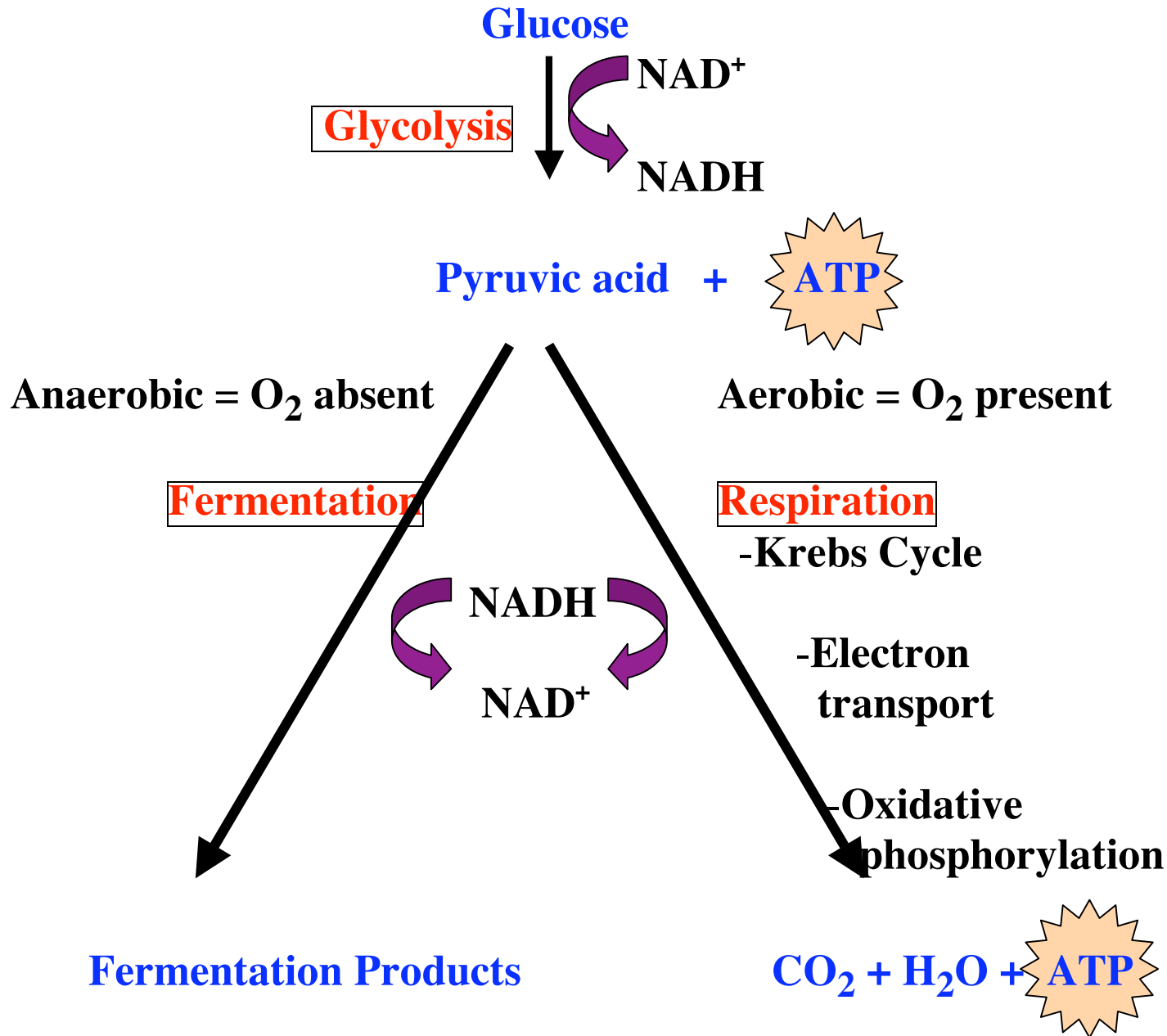
- Occurs if  $\text{O}_2$  is not present
- Regenerates  $\text{NAD}^+$  and disposes of electrons in organic waste products
- No energy is captured as ATP

## 3. Respiration

- Occurs if  $\text{O}_2$  is present as a terminal electron acceptor
- Completes oxidation of pyruvic acid, regenerates  $\text{NAD}^+$  and produces a lot more ATP

(Some microorganisms can use other compounds in place of  $\text{O}_2$ , Ex. nitrate, sulfate)

# Overview of Glucose Catabolism



## Fermentation Products

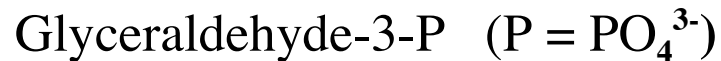
- **Organic acids**  
Ex. **lactic, acetic, butyric**
- **Alcohols**  
Ex. **ethanol, isopropyl**
- **Gases**  
Ex. **CO<sub>2</sub>, H<sub>2</sub>**

# Glycolysis

- Key steps

1. **2 ATPs used** to phosphorylate glucose

2. The product is split into two molecules of a 3-carbon compound:



3. Glyceraldehyde-3-P oxidized to pyruvic acid

- $\text{NAD}^+$  reduced to NADH

- **4 ATPs produced**

## Net Reaction of Glycolysis

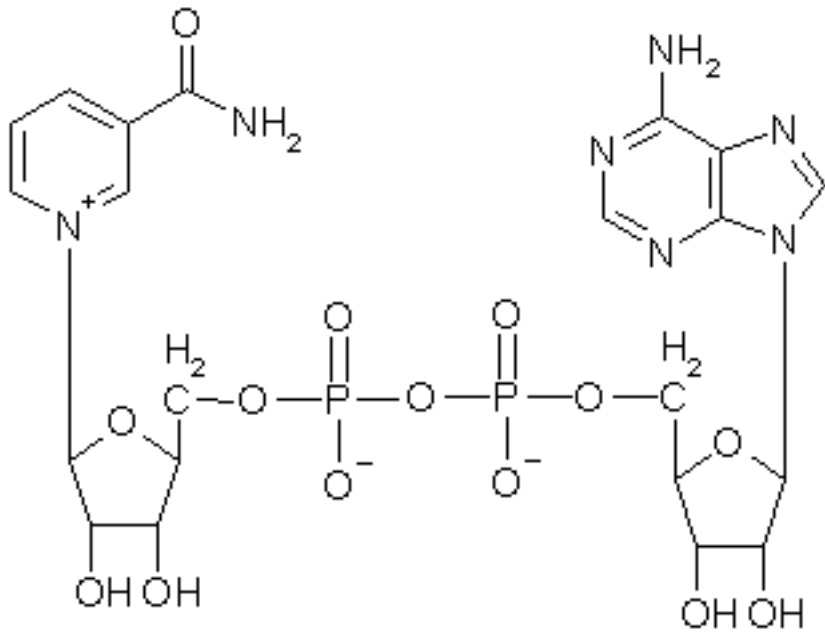


- $\text{NAD}^+$  is reduced to NADH by the electrons produced by oxidation of glucose
- Energy released from the oxidation reduction reactions is capture and stored as ATP

➤ For glycolysis to continue, NADH must now be oxidized to regenerate  $\text{NAD}^+$

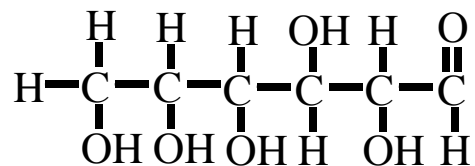
○ Two ways depending on whether  $\text{O}_2$  is absent or present

1. **Fermentation** (anaerobic environments) *or*
2. **Respiration** (aerobic environments)

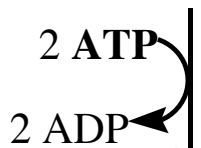


**NAD<sup>+</sup> (Nicotinamide adenine dinucleotide)**

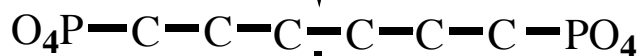
# Glucose



1.

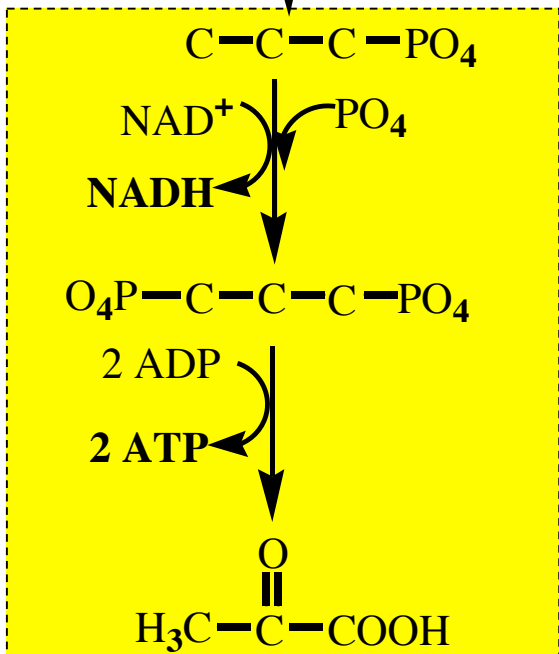
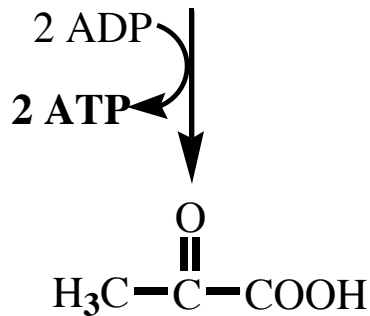
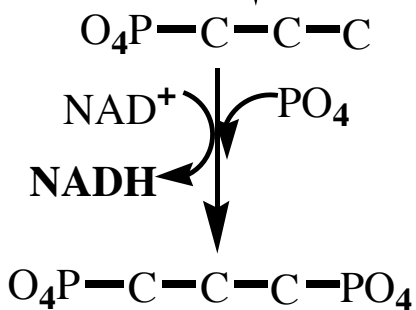


2.



## Glyceraldehyde-3-P

3.



## Pyruvic acid

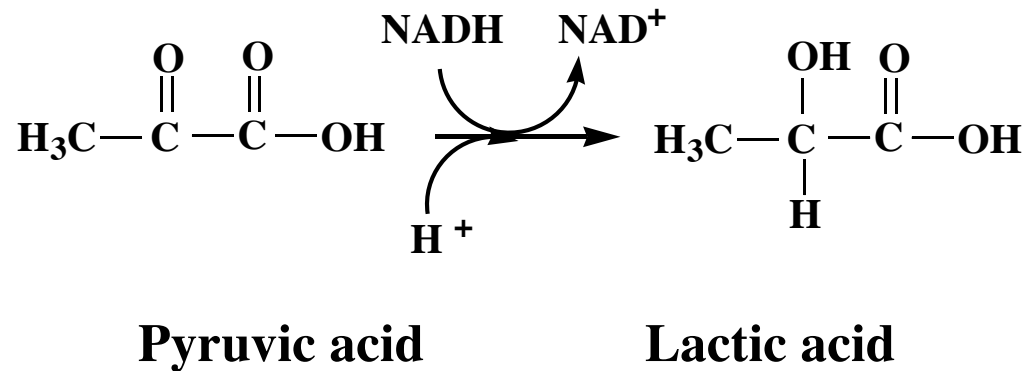
## Fermentation

- Purpose is to regenerate  $\text{NAD}^+$  so that glycolysis can continue when  $\text{O}_2$  is not present
  1.  $\text{NADH}$  is oxidized back into  $\text{NAD}^+$
  2. Pyruvic acid is reduced to **fermentation end products**
    - Organic acids
    - Alcohols
    - Gases:  $\text{CO}_2$  and  $\text{H}_2$
- The fermentation end products define the type of fermentation

## Types of fermentation

### 1. Homolactic acid fermentation (lactic acid only end product)

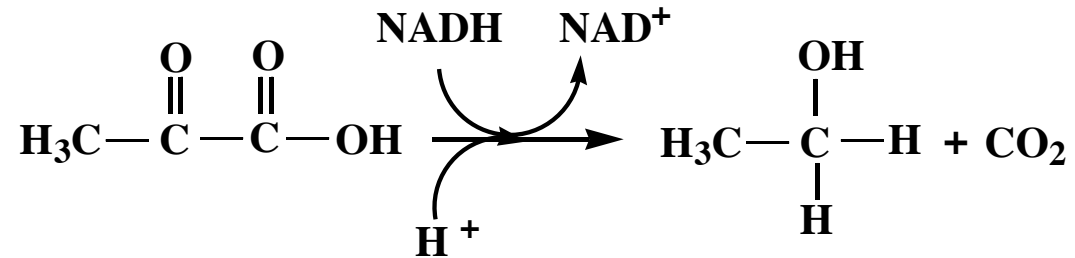
- Electrons are transferred from NADH to pyruvic acid



(NADH is oxidized/pyruvic acid is reduced)

- Lactic acid is a catabolic waste product (fermentation end product) used to dispose of electrons and regenerate  $\text{NAD}^+$ 
  - Lactic acid production lowers pH
- **Lactic acid bacteria** –Gram positive rods and cocci
  - Normal flora of vagina and intestinal tract
    - Prevents growth of pathogens
  - Used to make yogurt, sour cream, cheese, sauerkraut, silage

## 2. Alcoholic fermentation (ethanol and CO<sub>2</sub> end products)



**Pyruvic acid**

**Ethanol**

- Yeast used to make bread, wine, beer and ethanol for fuel
- Some bacteria also carry out alcoholic fermentation

### 3. Other types of fermentation (Fig. 8.26)

- Analysis of fermentation end products helps identify anaerobic microorganisms
  - Ex. Voges-Proskauer test for acetylmethylcarbinol (acetoin) is used to identify *Enterobacter* spp. –Gram negative, opportunistic pathogens

Figure 8.25

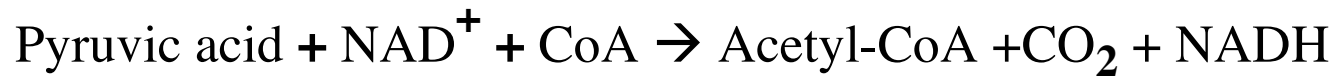
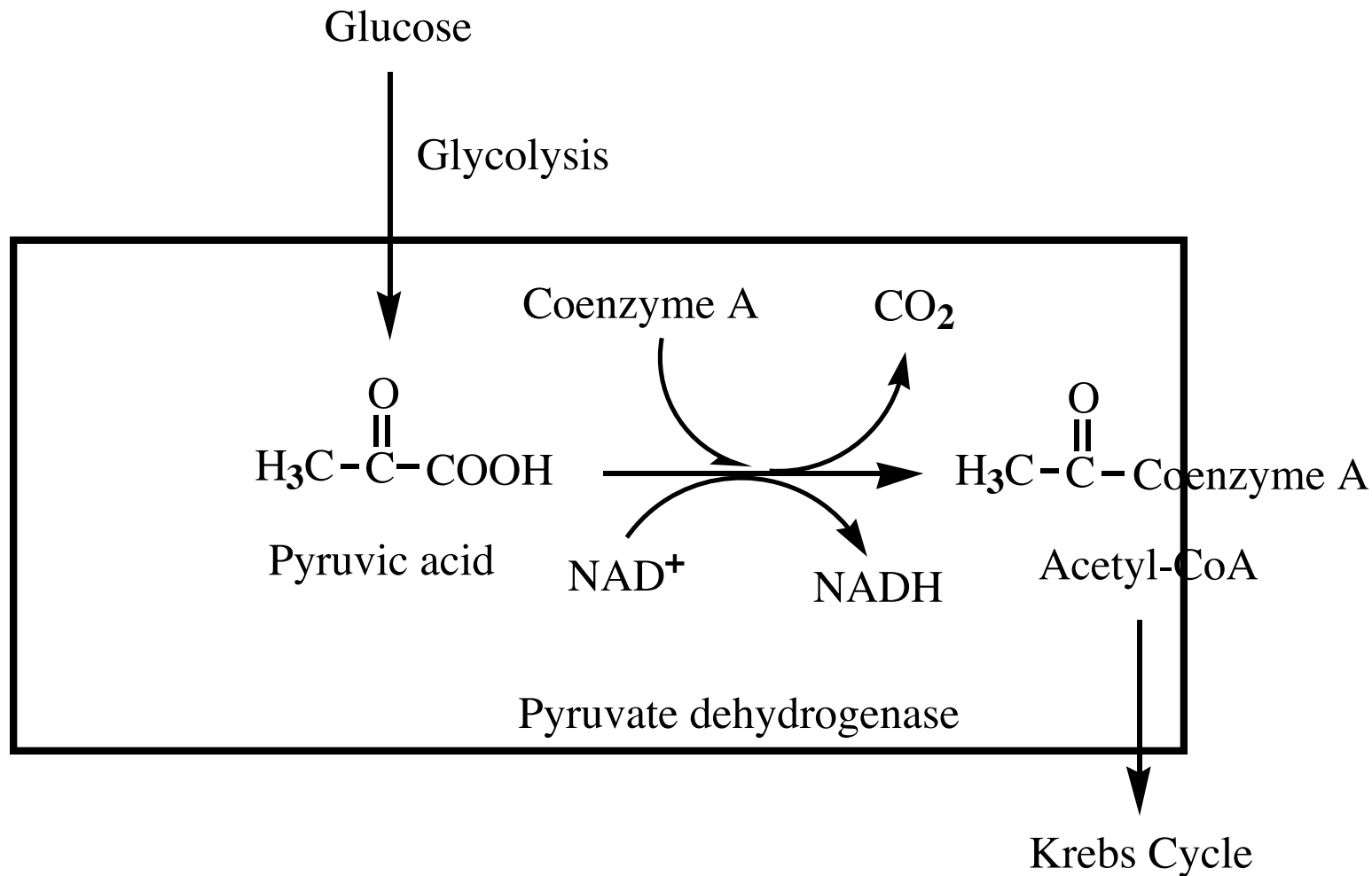
## Respiration (Aerobic)

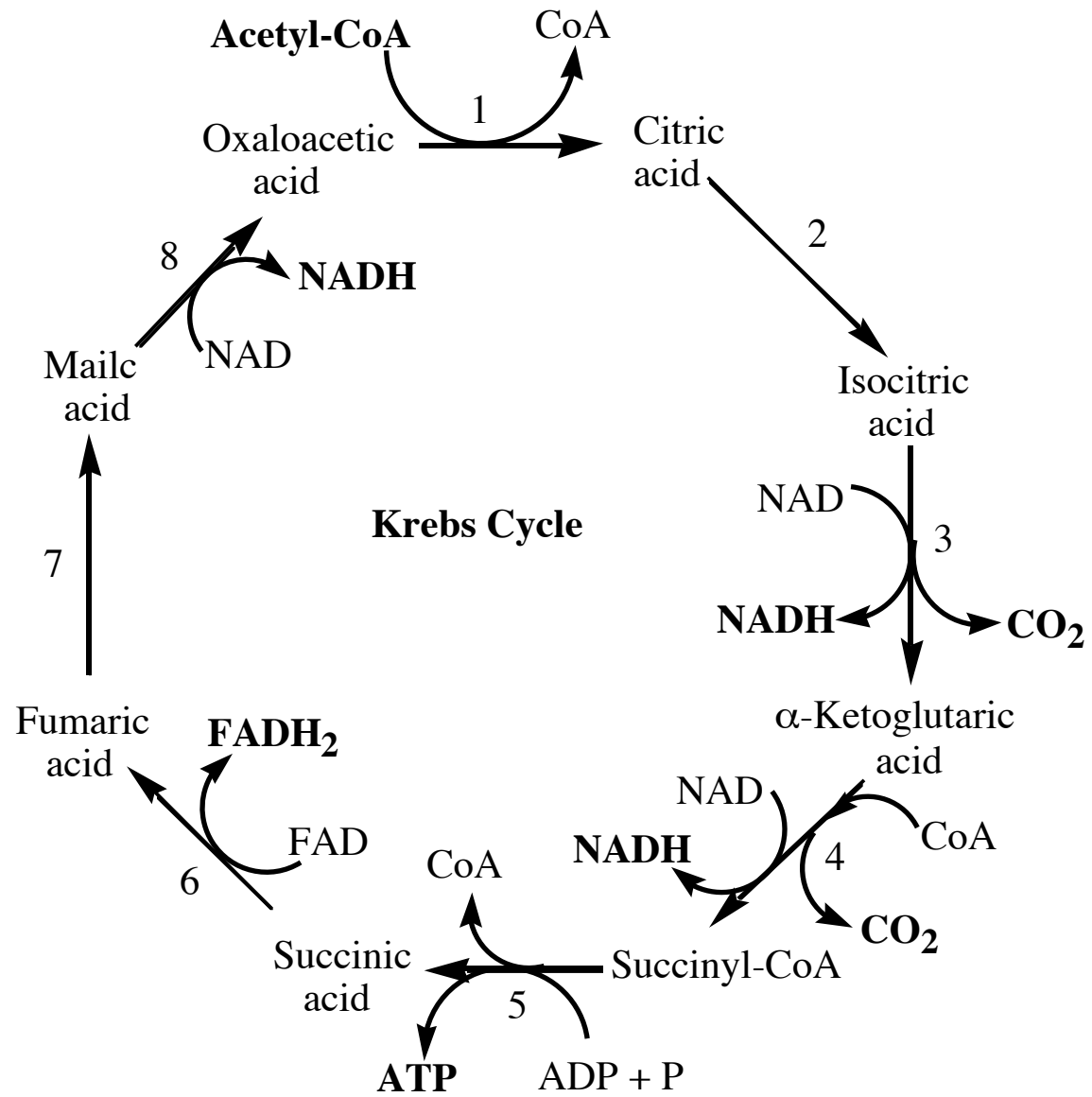
- Results in **complete oxidation** of carbon in glucose to  $\text{CO}_2$
- **Requires  $\text{O}_2$**  as electron acceptor
- Much **more ATP** is produced

➤ Following glycolysis, respiration of glucose involves four main processes:

1. **Oxidation of pyruvic acid** to acetyl-CoA and  $\text{CO}_2$
2. Oxidation of acetyl-CoA to  $\text{CO}_2$  via **Krebs cycle**
3. Regeneration of  $\text{NAD}^+$  and **transport of electrons** to  $\text{O}_2$
4. Formation of **ATP** via oxidative phosphorylation of ADP

# 1. Oxidation of pyruvic acid





## 2. Krebs Cycle (TCA Cycle)

- Each acetyl group is oxidized to 2 molecules of **CO<sub>2</sub>**
  - Electrons (4 pairs per acetyl group) from oxidation are transferred to 3 **NAD<sup>+</sup>** and 1 **FAD**
    - Three **NADH** and one **FADH<sub>2</sub>** are produced  
(FAD = flavin adenine dinucleotide)
  - Small amount of usable energy is produced (as **GTP** – animal cells or **ATP** – bacteria)
- Completes the oxidation of the 6 carbons of a glucose molecule to **CO<sub>2</sub>**
- **NAD<sup>+</sup>** and **FAD** must now be regenerated for the Krebs Cycle to continue
- Electrons from oxidation of **NADH** and **FADH<sub>2</sub>** are transported within the cell membrane to **O<sub>2</sub>**
  - Allows cell to capture a lot of energy as **ATP**

### 3. Electron transport

- **Electrons** derived from oxidation of NADH and FADH<sub>2</sub>
  - Regenerates the coenzymes for reuse in glycolysis and Krebs cycle
- Electrons are **transported** via a **chain** of protein carriers to O<sub>2</sub>
  - O<sub>2</sub> is **reduced** to water



- **Electron transport chain** located in a **membrane**
  - Cell membrane of prokaryotic cells
  - Mitochondrial membrane of eukaryotic cells
- **H<sup>+</sup> pumped** across membrane to **exterior** of bacterial cells
  - Results in **higher [H<sup>+</sup>]** outside cell than inside
  - The **concentration gradient** is a form of **potential energy**
    - Called the **proton motive force**
    - The energy is captured as **ATP** via **oxidative phosphorylation**

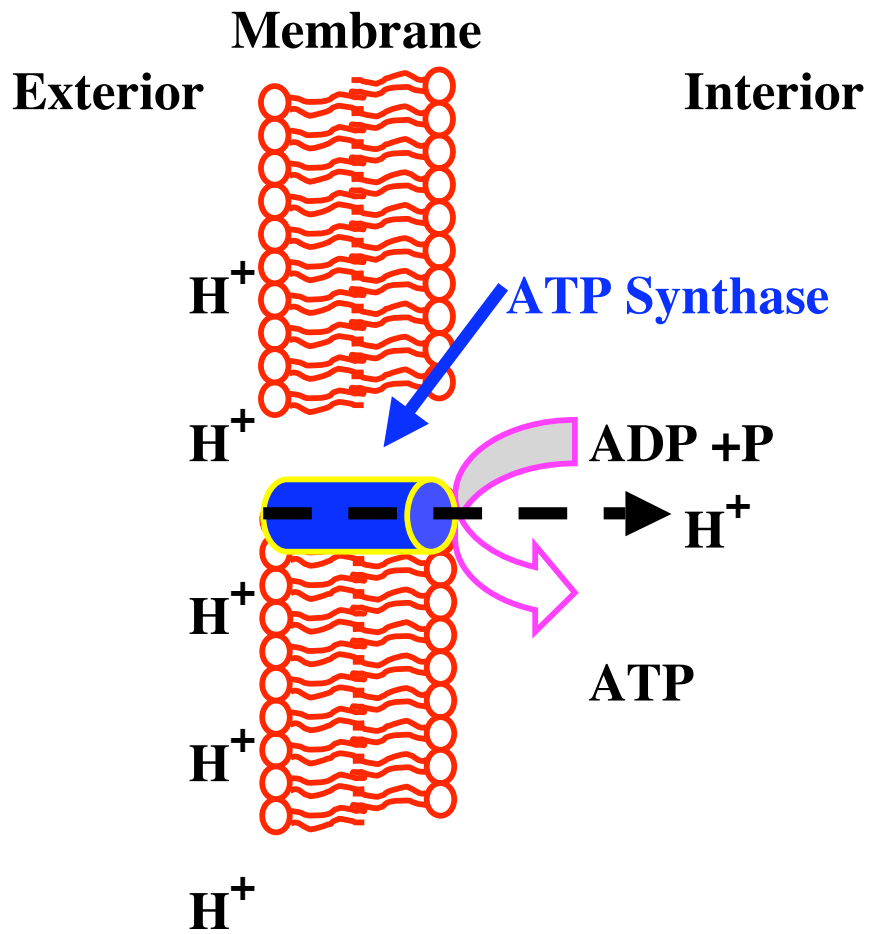
# Figure 8.22 Electron Transport and ATP Synthesis in Bacterial Cell

Figure 8.22

## Figure 8.21

## 4. Oxidative phosphorylation

- $H^+$  moves back across the membrane and into the cell via **chemiosmosis**
- ADP is phosphorylated to ATP
  - Reaction catalyzed by **ATP synthase**



## Figure 8.17

## Figure 8.23

## Glucose Fermentation vs. Aerobic Respiration

<u>Metabolic process</u>	<u>ATP Produced*</u>	
	<u>Fermentation</u>	<u>Respiration</u>
• Glycolysis	2	8
• Pyruvate to acetylCoA	-	6
• Krebs cycle	-	24
<b>Total</b>	<b>2</b>	<b>38</b>

**\*per molecule of glucose**

## Metabolism of fats and proteins

- Fats broken down into glycerol and fatty acids
  - Glycerol metabolized by glycolysis
  - Fatty acids broken down into acetyl-CoA which enters Krebs cycle
- Proteins broken down into amino acids
  - Amino group is removed
  - Rest of molecule enters glycolysis and is fermented or enters Krebs cycle

## Uses for ATP generated during catabolism

### 1. Biosynthesis (anabolism) --formation of covalent bonds

- Intermediate products of catabolic pathways can be diverted into biosynthetic pathways to produce new cell constituents
- Lipids, amino acids, enzymes, membrane proteins, nucleotides, DNA, RNA, peptidoglycan, etc.

### 2. Active transport of substances across cell membrane

### 3. Movement (motility)

Figure 8.26

## Figure 8.27