

# Environmental Microbiology

## Metabolic Pathways

prepared by Prof. Bulent Içgen

# Essentials of Catabolism

- Glycolysis
- Respiration and Electron Carriers
- The Proton Motive Force
- The Citric Acid Cycle
- Catabolic Diversity

# Catabolic Diversity

Microorganisms demonstrate a wide range of mechanisms for generating energy

- Fermentation
- Aerobic respiration
- Anaerobic respiration
- Chemolithotrophy
- Phototrophy

# Catabolic Diversity

## Anaerobic Respiration

- The use of electron acceptors other than oxygen
  - Examples include nitrate ( $\text{NO}_3^-$ ), ferric iron ( $\text{Fe}^{3+}$ ), sulfate ( $\text{SO}_4^{2-}$ ), carbonate ( $\text{CO}_3^{2-}$ ), certain organic compounds
- Less energy released compared to aerobic respiration
- Dependent on electron transport, generation of a proton motive force, and ATPase activity

# Catabolic Diversity

## Chemolithotrophy

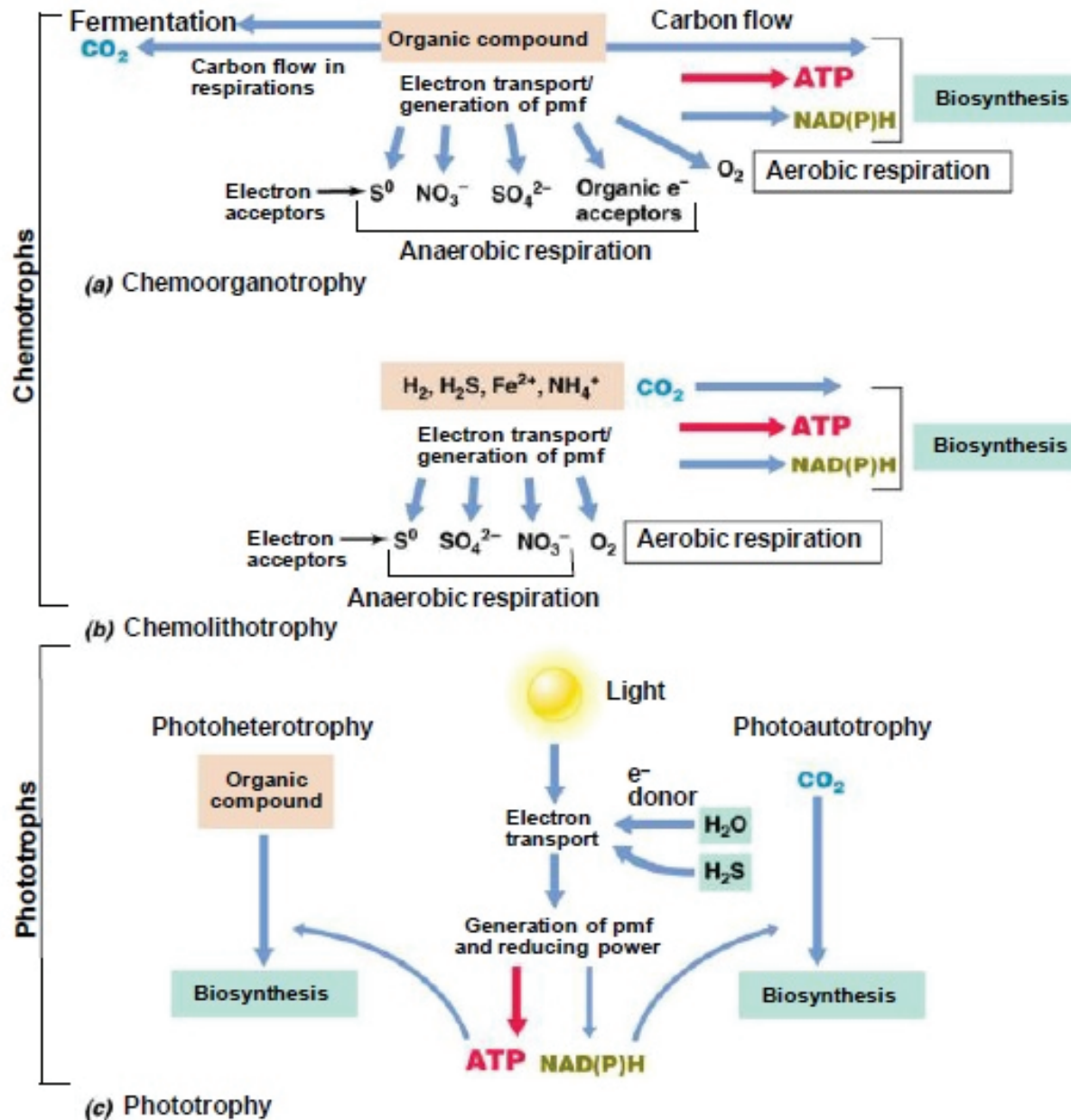
- Uses inorganic chemicals as electron donors
- Examples include hydrogen sulfide ( $\text{H}_2\text{S}$ ), hydrogen gas ( $\text{H}_2$ ), ferrous iron ( $\text{Fe}^{2+}$ ), ammonia ( $\text{NH}_3$ )
- Typically aerobic
- Begins with oxidation of inorganic electron donor
- Uses electron transport chain and proton motive force
- Autotrophic; uses  $\text{CO}_2$  as carbon source

# Catabolic Diversity

**Phototrophy:** uses light as energy source

- Photophosphorylation: light-mediated ATP synthesis
- Photoautotrophs: use ATP for assimilation of CO<sub>2</sub> for biosynthesis
- Photoheterotrophs: use ATP for assimilation of organic carbon for biosynthesis

# Catabolic Diversity



Chemoorganotrophs differ from chemolithotrophs in two important ways:

1. The nature of the electron donor (organic vs. inorganic compounds, respectively) and
2. The nature of the source of cellular carbon (organic compounds vs. CO<sub>2</sub> respectively).

However, note the importance of electron transport driving proton motive force formation in all forms of respiration and in photosynthesis.

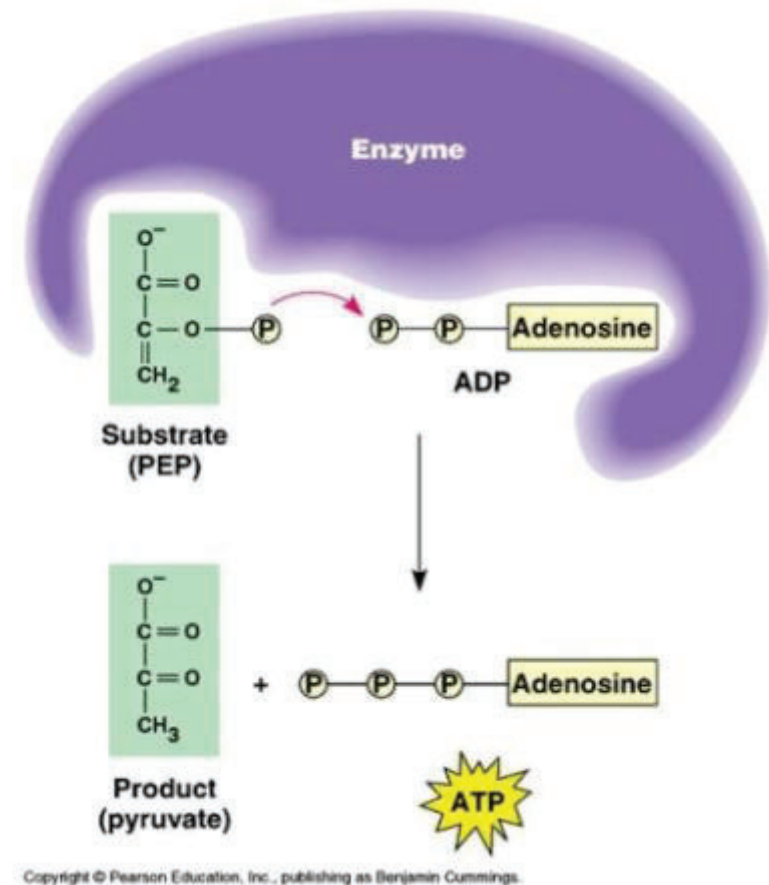
# Glycolysis

- Two reaction series are linked to energy conservation in chemoorganotrophs: fermentation and respiration
- Differ in mechanism of ATP synthesis
- Fermentation: **substrate-level phosphorylation**; ATP directly synthesized from an energy-rich intermediate
- Respiration: **oxidative phosphorylation**; ATP produced from proton motive force formed by transport of electrons

# ATP Synthesis = Phosphorylation

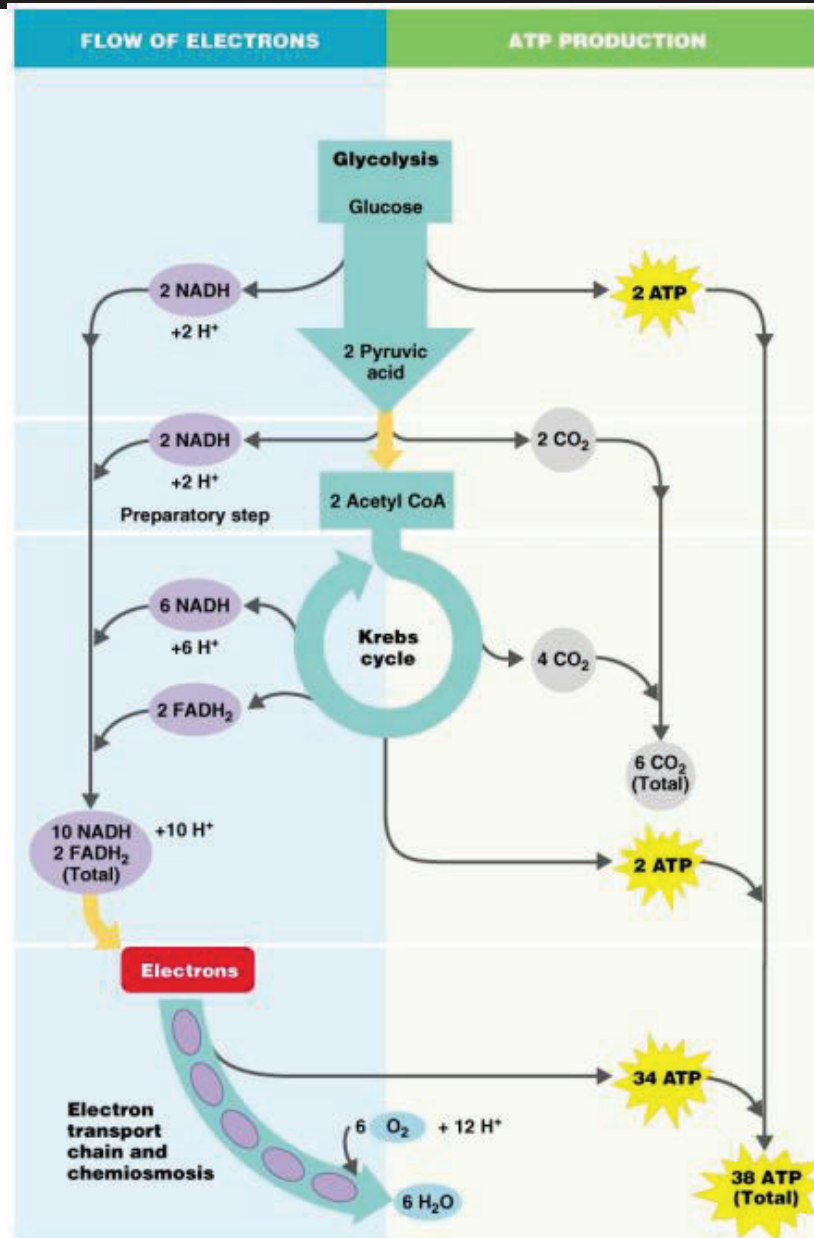
## 3 types of phosphorylation:

- Substrate Level Phosphorylation
  - ATP synthesis during glycolysis
- Oxidative Phosphorylation
  - ATP synthesis during electron transport chain
- Photophosphorylation
  - ATP synthesis during photosynthesis



# Metabolism for Energy Production

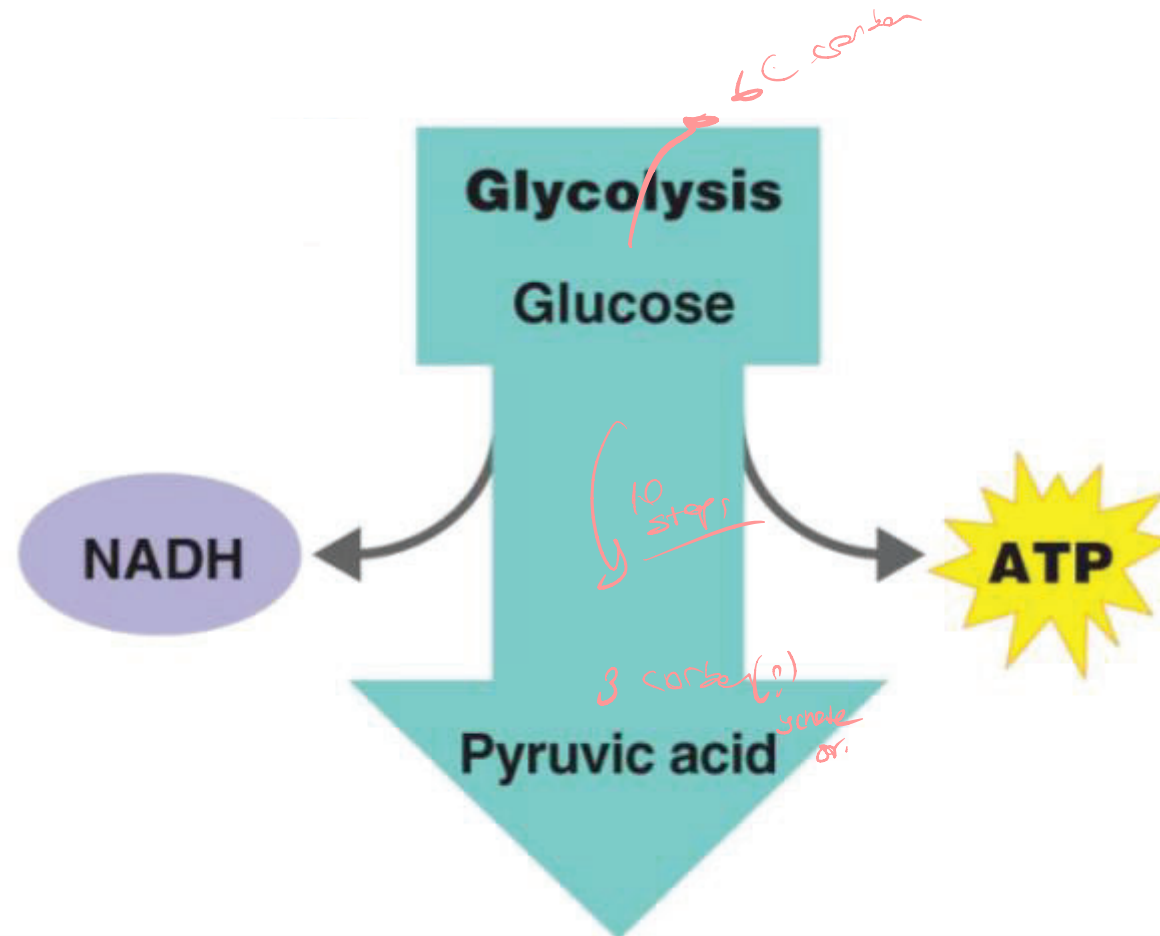
- Energy production involves a series of oxidation-reduction reactions to slowly release the energy stored in organic molecules
  - if not released in a slow stepwise process too much energy would be lost to heat and destroy the cell/organism
- Carbohydrate catabolism by microorganisms involves 2 general processes cellular respiration and/or fermentation
  - *Glycolysis* (part of cellular respiration & fermentation)
  - *Krebs cycle*
  - *Electron transport chain*



$1 \text{ NADH} = 2 \text{ ATP}$       $1 \text{ FADH} = 2 \text{ ATP}$   
 intermediat step  $\rightarrow$  very productive  
 creb cycle  $\rightarrow$  highly productive  
 in terms of energy.  
 2 ATP  $\rightarrow$  produced substrate  
 level.

# Glycolysis (Embden-Meyerhof Pathway)

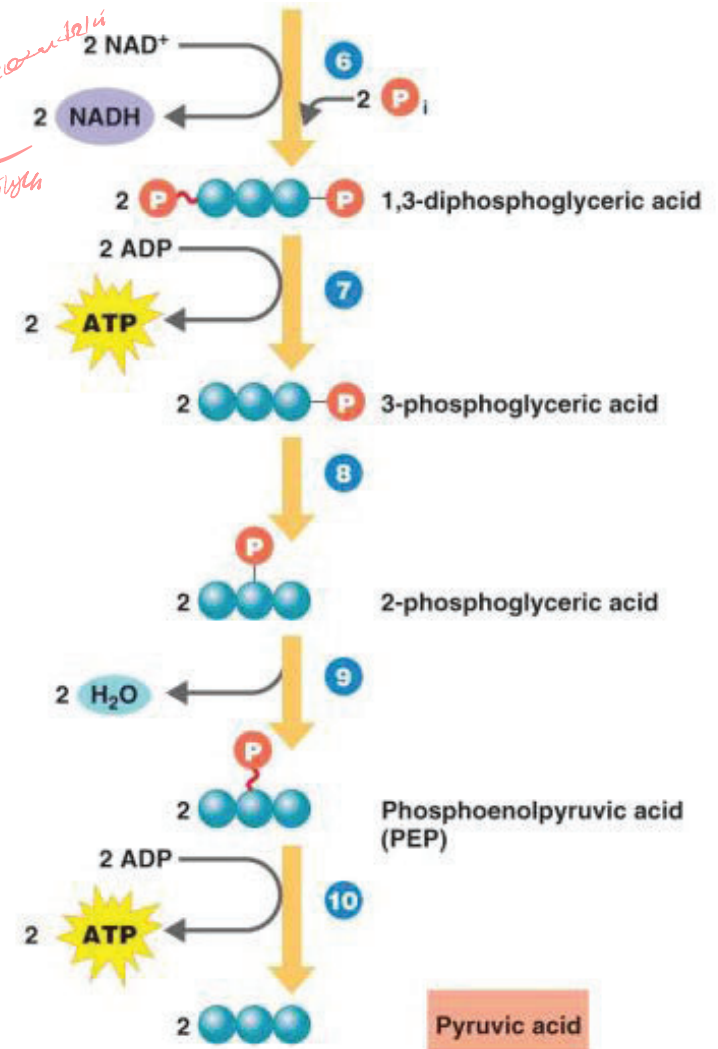
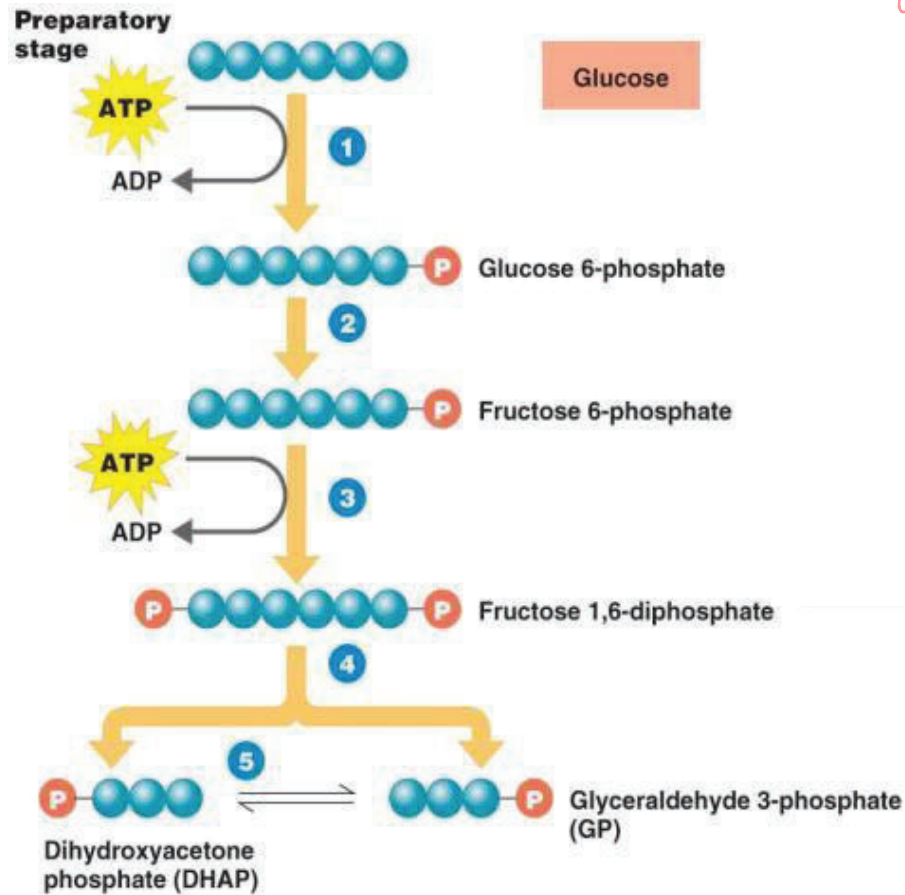
- The oxidation of glucose to pyruvic acid (aka pyruvate) produces ATP and NADH



# Glycolysis

- Total NET production of energy in Glycolysis:
  - 2 ATP
  - 2 NADH

*1 step → cell burn  
duscu  
cunici  
rodici  
cok  
yuzen*

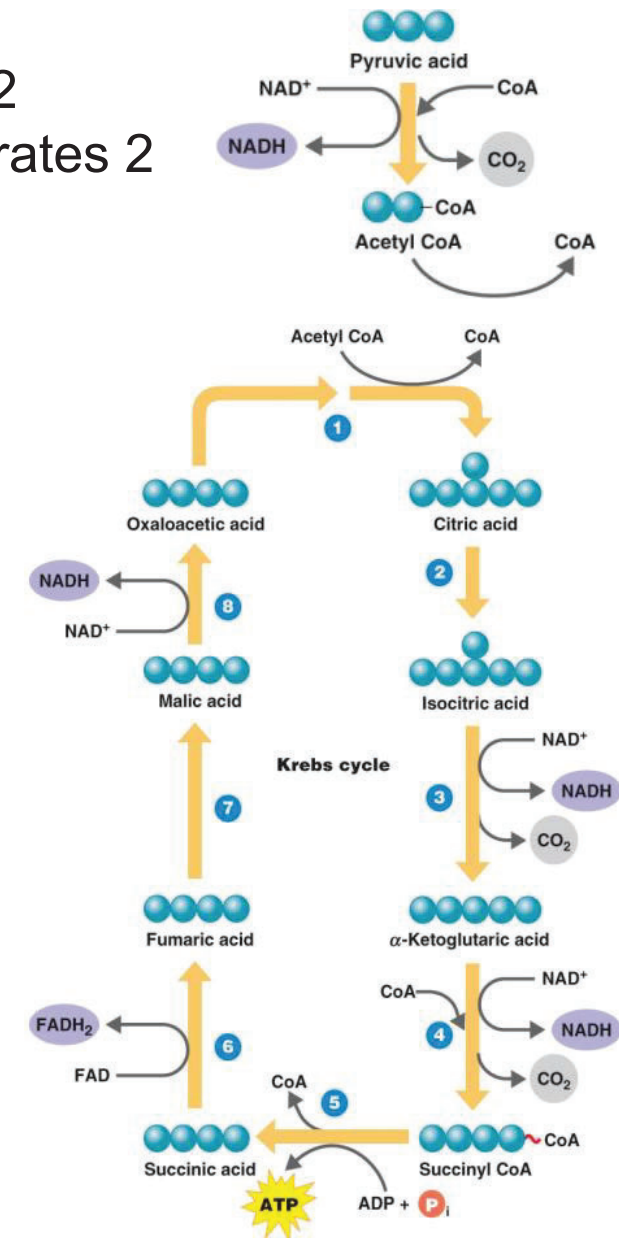


# Microbial Alternatives to Glycolysis

- Pentose phosphate pathway
  - uses pentoses (5 carbon sugars) and NADPH
  - operates simultaneously with glycolysis in some bacteria
  - occurs in the cytosol
  - *Bacillus subtilis*, *E. coli*, *Leuconostoc mesenteroides* & *Enterococcus faecalis*
- Entner-Doudoroff pathway
  - an alternate pathway that catabolizes glucose to pyruvate using different enzymes than glycolysis and the pentose phosphate pathway
  - produces NADPH and ATP like glycolysis
  - gram (-) bacteria – *Pseudomonas*, *Rhizobium*, *Agrobacterium*

# Krebs Cycle

- The 2 pyruvate from glycolysis are converted to 2 acetyl CoA in an intermediate reaction that generates 2 NADH
- For every 2 acetyl CoA that enter the Krebs cycle, 6 molecules of NADH, 2 FADH & 2 ATP are produced
- Many of the intermediates in the Krebs cycle also play a role in other pathways like amino acid biosynthesis
- End result of the metabolism of one molecule of glucose after glycolysis, Krebs & electron transport is 38 ATP (36 for eukaryotic organisms)



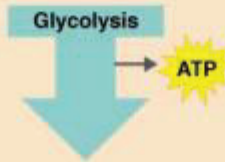
**Table 5.3 ATP Yield during Prokaryotic Aerobic Respiration of One Glucose Molecule**

**Source**

**ATP Yield (Method)**

**Glycolysis**

1. Oxidation of glucose to pyruvic acid
2. Production of 2 NADH



2 ATP (substrate-level phosphorylation)

6 ATP (oxidative phosphorylation in electron transport chain)

**Preparatory Step**

1. Formation of acetyl CoA produces 2 NADH



6 ATP (oxidative phosphorylation in electron transport chain)

**Krebs Cycle**

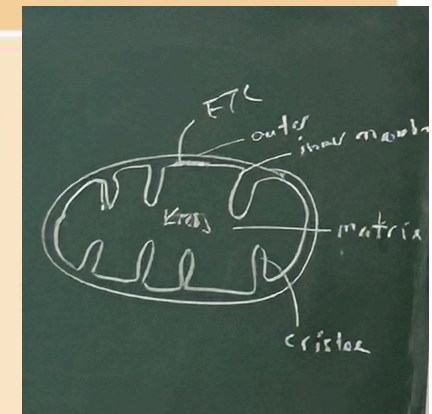
1. Oxidation of succinyl CoA to succinic acid
2. Production of 6 NADH
3. Production of 2 FADH

2 GTP (equivalent of ATP; substrate-level phosphorylation)

18 ATP (oxidative phosphorylation in electron transport chain)

4 ATP (oxidative phosphorylation in electron transport chain)

Total: 38 ATP



*prokaryotic Eukaryotic = 38 ATP*

*celling = ? Some of the energy leaks from mitochondria during the transfer*

# Anaerobic Respiration

- Anaerobic Respiration: General Principles
- Nitrate Reduction and Denitrification
- Sulfate and Sulfur Reduction
- Acetogenesis
- Methanogenesis
- Proton Reduction
- Other Electron Acceptors
- Anoxic Hydrocarbon Oxidation Linked to Anaerobic Respiration

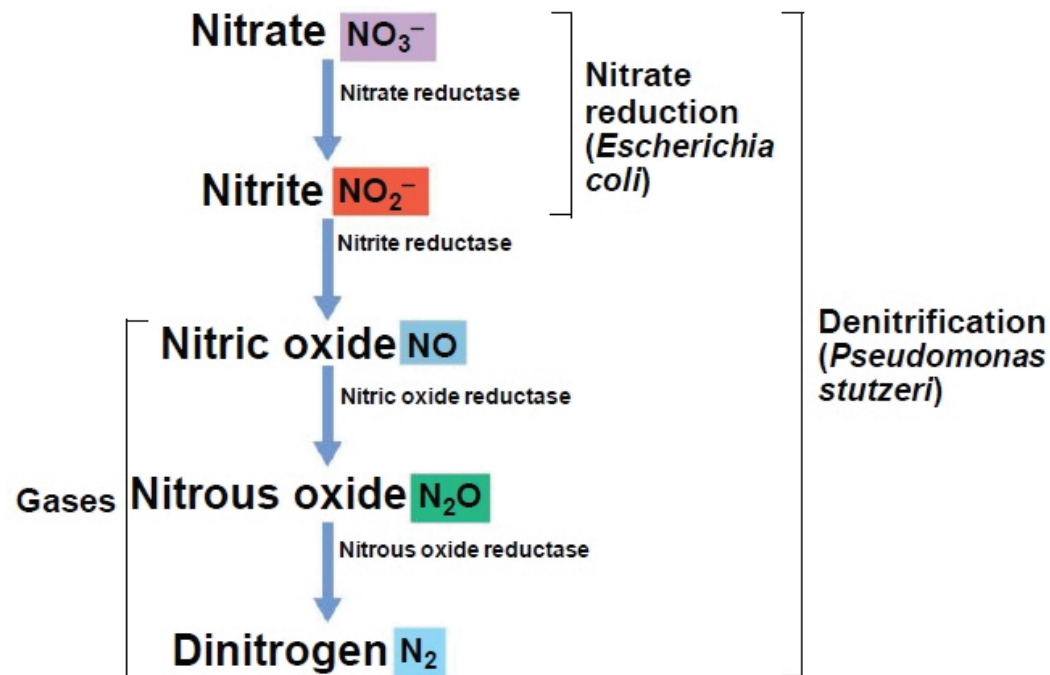
# Anaerobic Respiration: General Principles

- In anaerobic respiration, electron acceptors other than  $O_2$  are used
- Anaerobic and aerobic respiratory systems are similar
- But anaerobic respiration yields less energy than aerobic respiration
- Energy released from redox reactions can be determined by comparing reduction potentials
- In the assimilative metabolism of an inorganic compound (e.g.,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $CO_2$ ) the reduced compounds are used in biosynthesis
- During anaerobic respiration, the reduction of inorganic compounds is called dissimilative metabolism because the reduced products are excreted

# Nitrate Reduction and Denitrification

- Inorganic nitrogen compounds are the most common electron acceptors in anaerobic respiration
- All products of nitrate reduction (denitrification) are gaseous
- Denitrification is the main biological source of gaseous  $N_2$
- Enzymes of the pathway are repressed by oxygen

Steps in the dissimilative reduction of nitrate



# Sulfate and Sulfur Reduction

- Inorganic sulfur compounds can be used as electron acceptors in anaerobic respiration
- Reduction of  $\text{SO}_4^{2-}$  to  $\text{H}_2\text{S}$  proceeds through several intermediates and requires activation of sulfate by ATP.

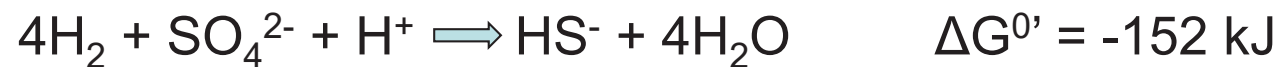
**Table 14.6** Sulfur compounds and electron donors for sulfate reduction

Compound	Oxidation state of S atom
<b>Oxidation states of key sulfur compounds</b>	
Organic S (R—SH)	-2
Sulfide ( $\text{H}_2\text{S}$ )	-2
Elemental sulfur ( $\text{S}^0$ )	0
Thiosulfate ( $-\text{S}-\text{SO}_3^{2-}$ )	-2/+6
Sulfur dioxide ( $\text{SO}_2$ )	+4
Sulfite ( $\text{SO}_3^{2-}$ )	+4
Sulfate ( $\text{SO}_4^{2-}$ )	+6
<b>Some electron donors used for sulfate reduction</b>	
$\text{H}_2$	Acetate
Lactate	Propionate
Pyruvate	Butyrate
Ethanol and other alcohols	Long-chain fatty acids
Fumarate	Benzoate
Malate	Indole
Choline	Various hydrocarbons

# Sulfate and Sulfur Reduction

- Many different compounds can serve as electron donors in sulfate reduction

Examples: H<sub>2</sub>, organic compounds, phosphite

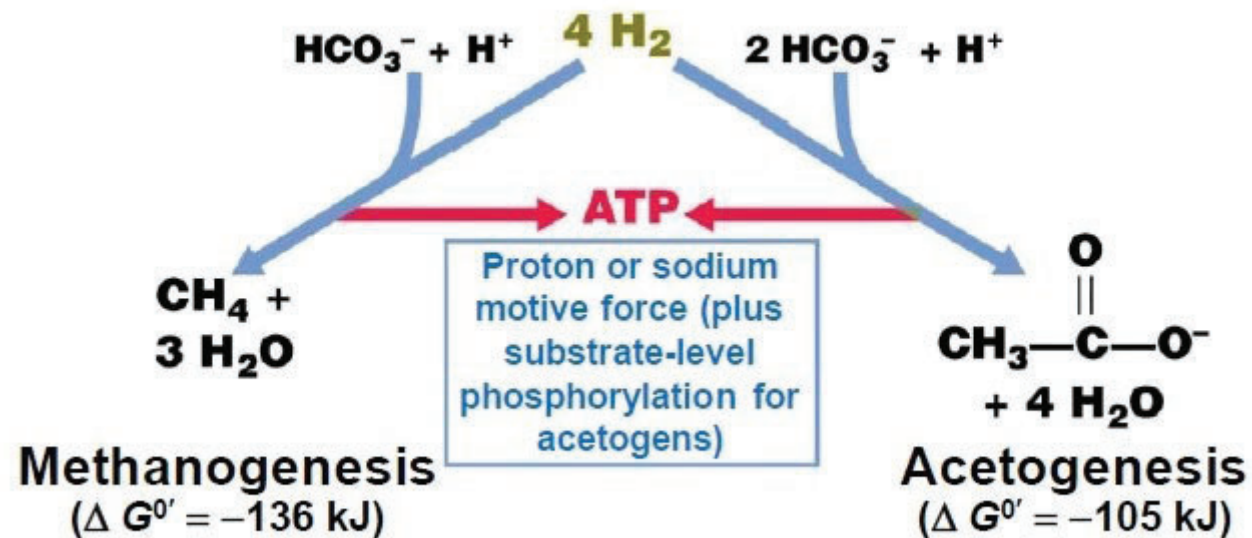


# Acetogenesis

- Acetogens and methanogens use  $\text{CO}_2$  as an electron acceptor in anaerobic respiration
- $\text{H}_2$  is the major electron donor for both groups of organisms
- Acetogens carry out the reaction



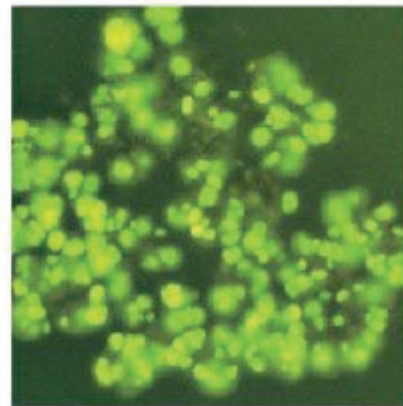
The contrasting processes of methanogenesis and acetogenesis



# Methanogenesis

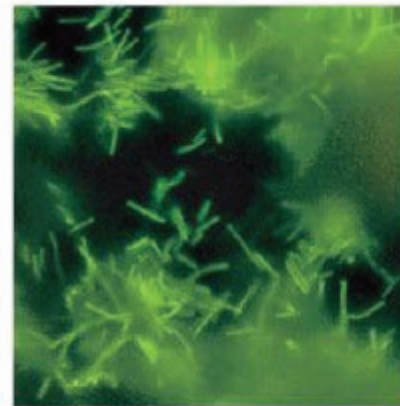
Biological production of methane

- Carried out by a group of strictly anaerobic
- Archaea called the methanogens
- Involves a complex series of biochemical reactions that use novel coenzymes
- The autofluorescence of coenzyme  $F_{420}$  can be used to identify methanogens microscopically



T. D. Brock

(a) *Methanosarcina barkeri*



T. D. Brock

(b) *Methanobacterium formicicum*

Fluorescence due to the methanogenic coenzyme  $F_{420}$ . The organisms were made visible by excitation with blue light in a fluorescence microscope

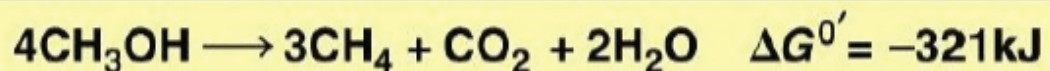
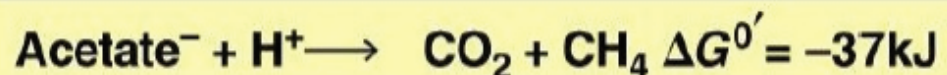
# Methanogenesis

- H<sub>2</sub> is the major electron donor for methanogenesis (Figure 14.20)



$$\Delta G^{0'} = -130.7 \text{ kJ/reaction}$$

- Additional electron donors exist
  - Examples: formate, CO, organic compounds



# Proton Reduction

- *Pyrococcus furiosus*

- Member of the Archaea
- Grows optimally at 100°C on sugars and small peptides as electron donors
- May have the simplest anaerobic respiration mechanism
- Organism uses modified glycolysis and protons in anaerobic respiration linked to ATPase activity



# Other Electron Acceptors

- The reduction of arsenate has been employed for cleanup of toxic wastes and groundwater
- Halogenated compounds can also serve as electron acceptors via a process called reductive dechlorination (dehalorespiration)

Biomining during arsenate reduction by the sulfate-reducing bacterium  
*Desulfotomaculum auripigmentum*

*Desulfotomaculum* can reduce  $\text{AsO}_4^{3-}$  to  $\text{AsO}_3^{3-}$ , along with sulfate ( $\text{SO}_4^{2-}$ ) to sulfide ( $\text{HS}^-$ )



Dianne K. Newman  
and Stephen Tay

Appearance of  
culture bottle after  
inoculation

Synthetic sample  
of  $\text{As}_2\text{S}_3$

Following growth for 2 weeks  
and biomineralization of  
arsenic trisulfide,  $\text{As}_2\text{S}_3$

# Anoxic Hydrocarbon Oxidation

- Aliphatic and aromatic hydrocarbons and organic compounds containing only carbon and hydrogen can be oxidized anaerobically
- The first step in degradation is the addition of oxygen to the molecule through the incorporation of fumarate
- Hydrocarbons are oxidized to intermediates that can be catabolized via the citric acid cycle
- Aliphatic hydrocarbons are straight-chain saturated or unsaturated compounds
- Many of them are substrates for denitrifying and sulfate-reducing bacteria
- Aromatic hydrocarbons are catabolized by ring reduction and cleavage
- Can be degraded by some denitrifying, ferric iron-reducing, and sulfate-reducing bacteria

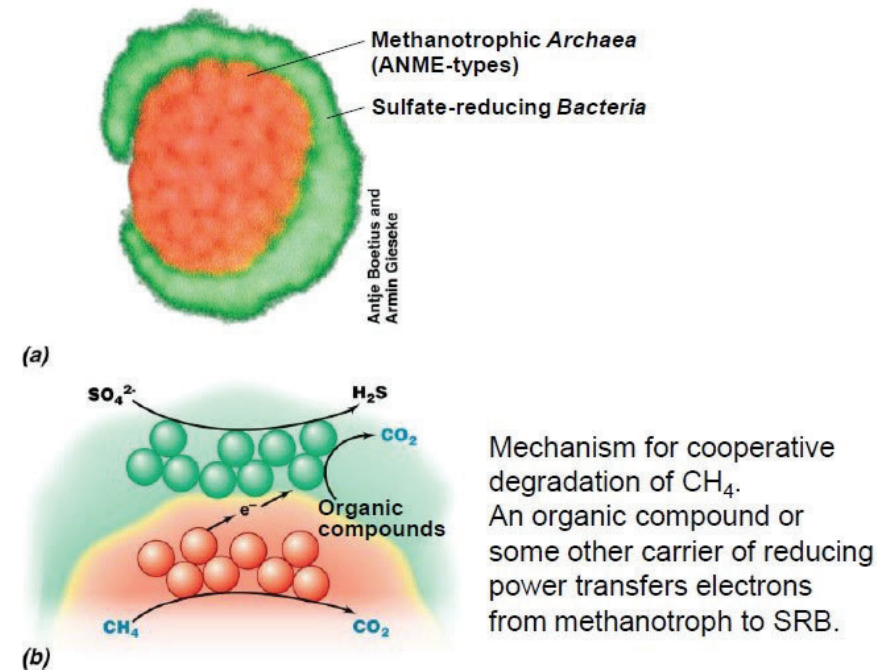
# Anoxic Hydrocarbon Oxidation

## Methane

- The simplest hydrocarbon
- Can be oxidized under anoxic conditions by a consortia containing sulfate-reducing bacteria and methanotrophic archaea



Anoxic methane oxidation



# Fermentation Definitions

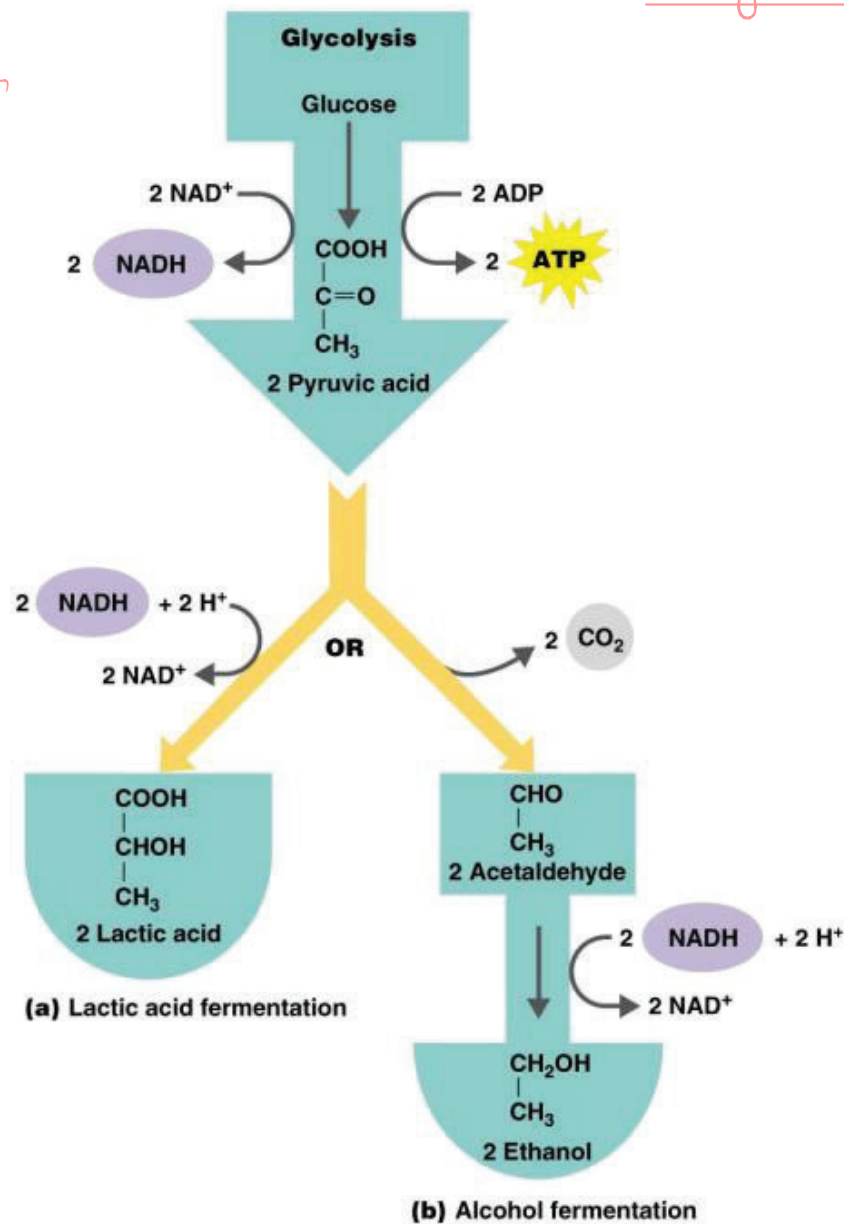
- Any spoilage of food by microorganisms (general use)
- Any process that produces alcoholic beverages or acidic dairy products (general use)
- Any large-scale microbial process occurring with or without air (common definition used in industry)
- Scientific definition:
  - releases energy from oxidation of organic molecules
  - does not require oxygen
  - does not use the Krebs cycle or ETC
  - uses an organic molecule as the final electron acceptor

fermentative bacteria

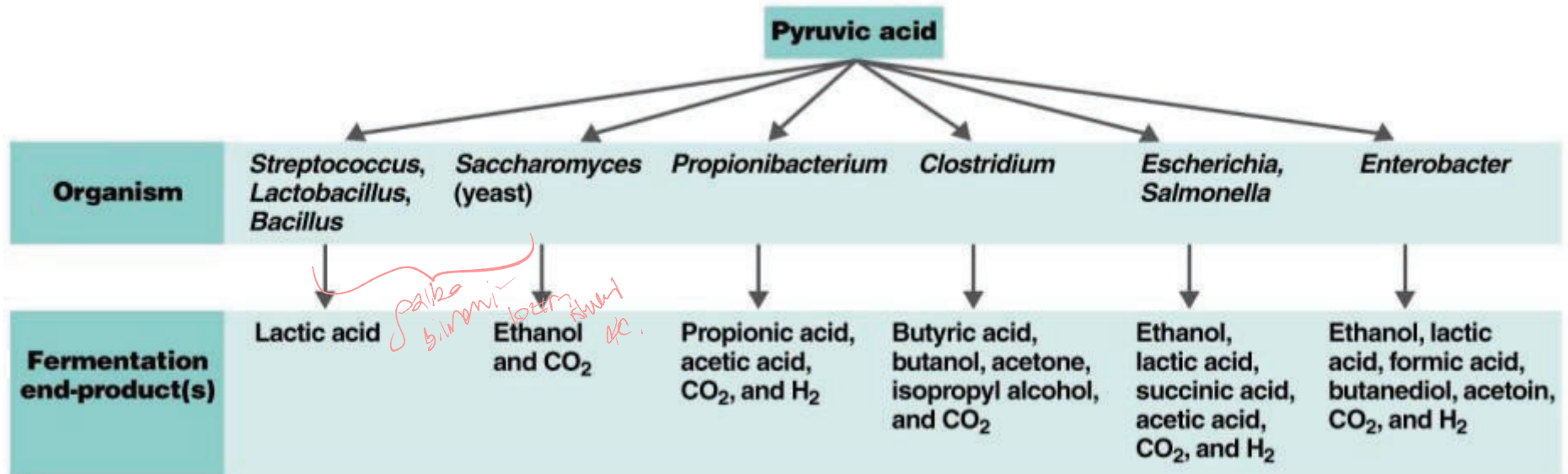
can go through oxidative fermentation

no electron stop

only 2ATP



# End-Products of Fermentation



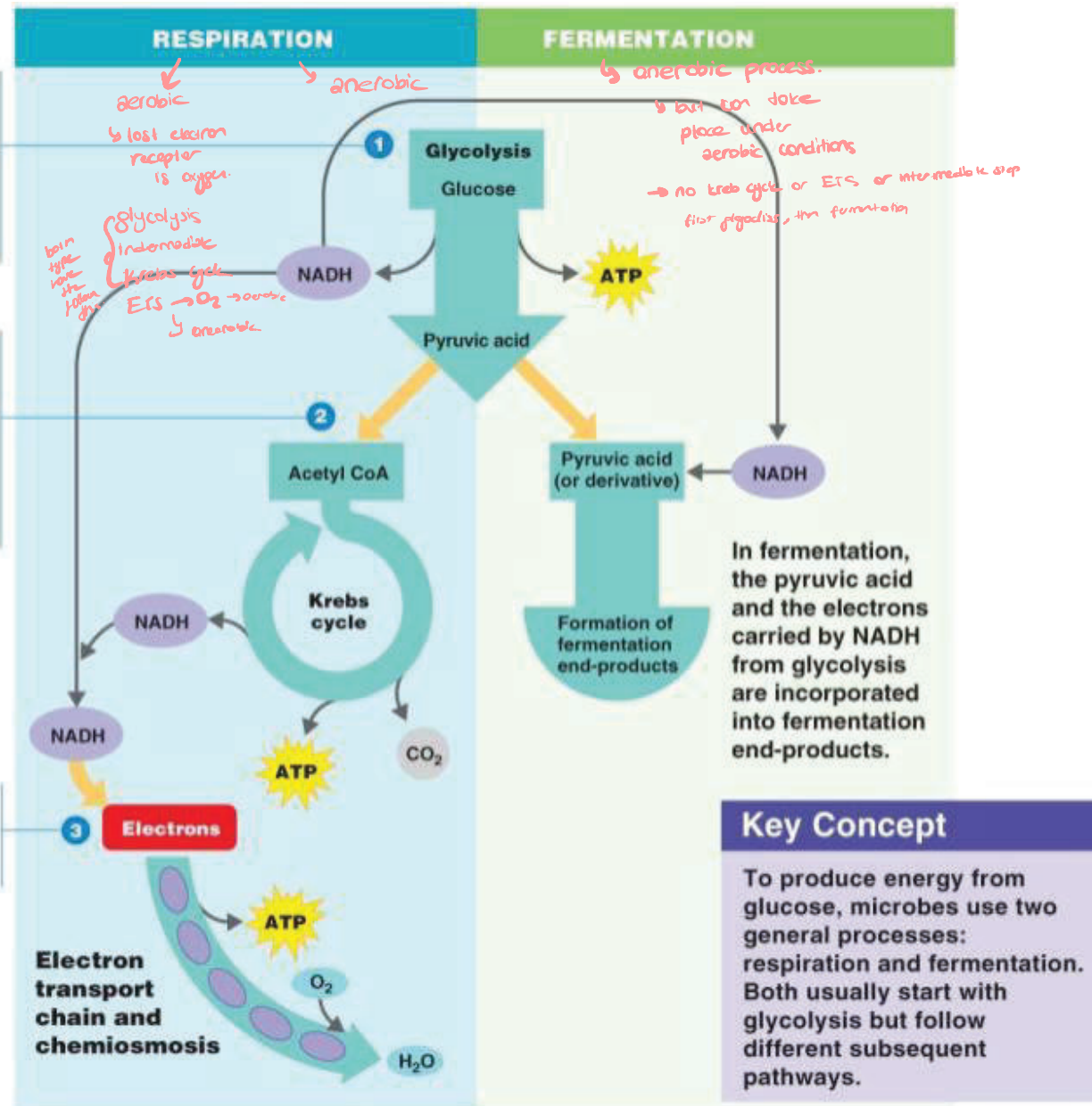
(b)

# Overview of Respiration & Fermentation

**1** Glycolysis produces ATP and reduces  $\text{NAD}^+$  to  $\text{NADH}$  while oxidizing glucose to pyruvic acid. In respiration, the pyruvic acid is converted to the first reactant in the Krebs cycle.

**2** The Krebs cycle produces ATP and reduces  $\text{NAD}^+$  (and another electron carrier called  $\text{FADH}_2$ ) while giving off  $\text{CO}_2$ . The  $\text{NADH}$  and  $\text{FADH}_2$  from both processes carry electrons to the electron transport chain.

**3** In the electron transport chain, the energy of the electrons is used to produce a great deal of ATP.

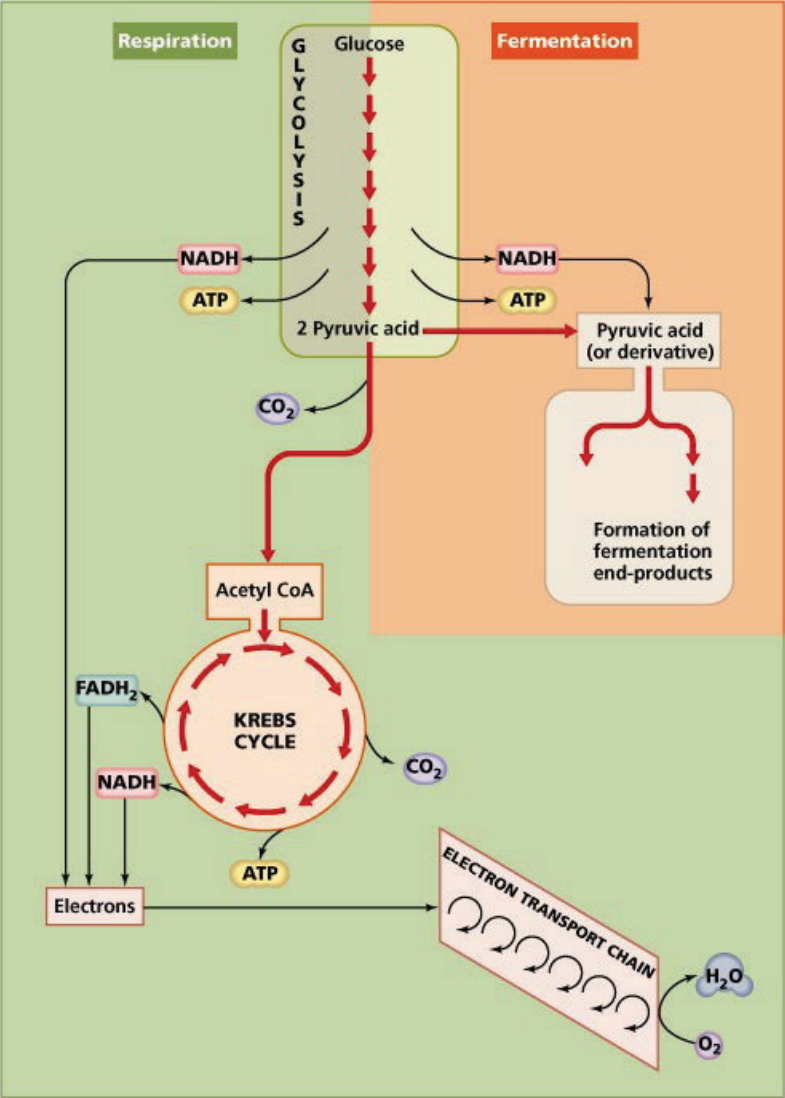


**Key Concept**

To produce energy from glucose, microbes use two general processes: respiration and fermentation. Both usually start with glycolysis but follow different subsequent pathways.

# Carbohydrate Catabolism

- The breakdown of carbohydrates to release energy
  - Glycolysis
  - Krebs cycle
  - Electron transport chain



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# Alternatives to Glycolysis

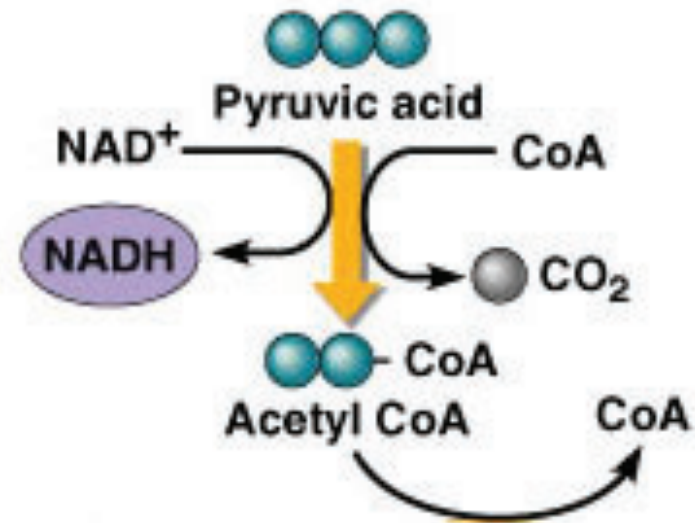
- **Pentose phosphate pathway:**
  - Uses pentoses and NADPH
  - Operates with glycolysis
- **Entner-Doudoroff pathway:**
  - Produces NADPH and ATP
  - Does not involve glycolysis
  - *Pseudomonas, Rhizobium, Agrobacterium*

# Cellular Respiration

- Oxidation of molecules liberates electrons for an electron transport chain
- ATP generated by oxidative phosphorylation

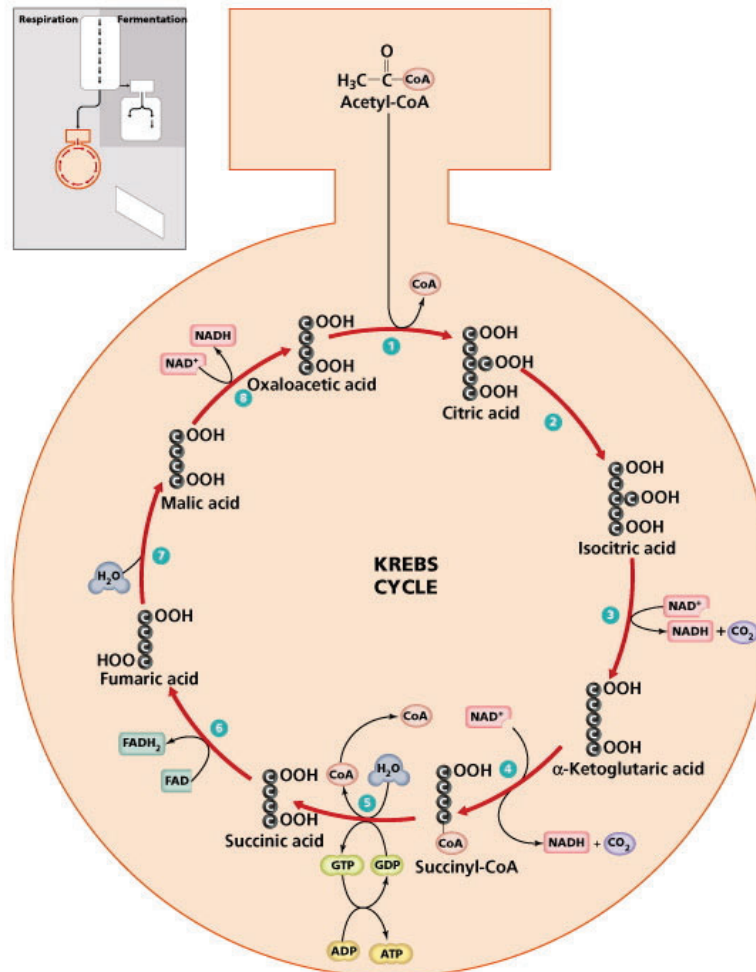
# Intermediate Step

- Pyruvic acid (from glycolysis) is oxidized and decarboxylated



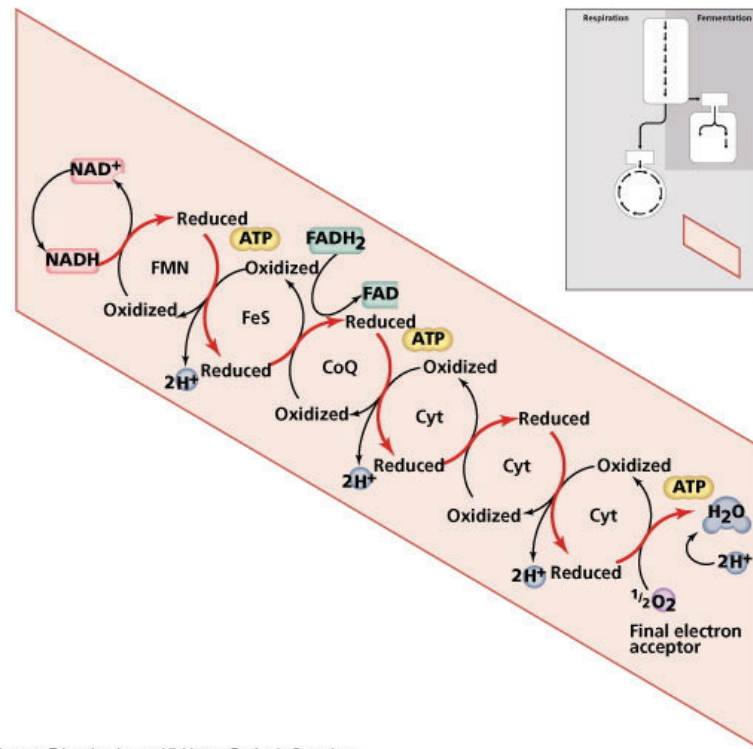
# Krebs Cycle

- Oxidation of acetyl CoA produces NADH and FADH<sub>2</sub>



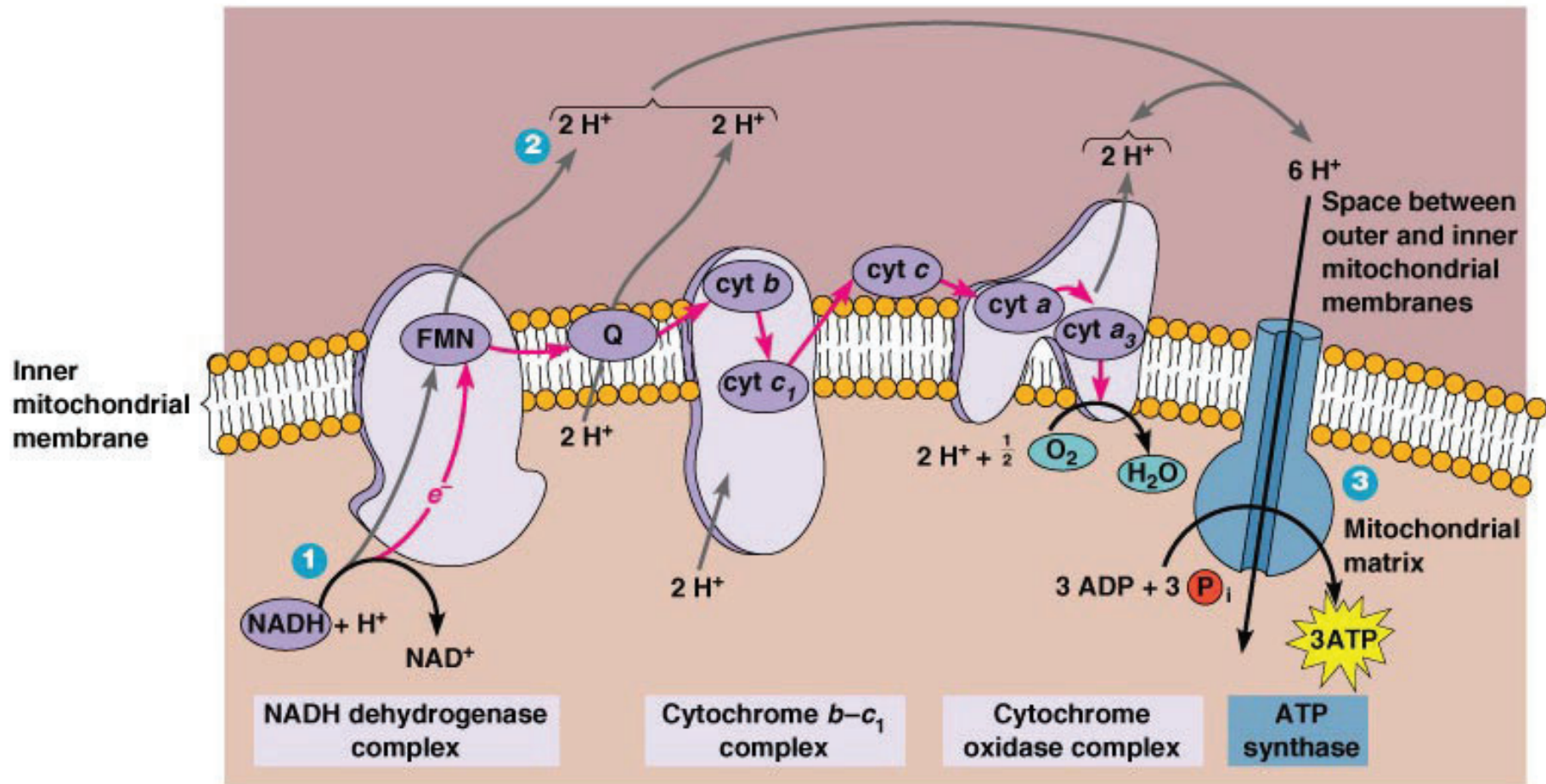
# The Electron Transport Chain

- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain.
- Energy released can be used to produce ATP by chemiosmosis.



<b>Pathway</b>	<b>Eukaryote</b>	<b>Prokaryote</b>
Glycolysis	Cytoplasm	Cytoplasm
Intermediate step	Cytoplasm	Cytoplasm
Krebs cycle	Mitochondrial matrix	Cytoplasm
ETC	Mitochondrial inner membrane	Plasma membrane

# Chemiosmosis



- Energy produced from complete oxidation of 1 glucose using aerobic respiration

<b>Pathway</b>	<b>ATP produced</b>	<b>NADH produced</b>	<b>FADH<sub>2</sub> produced</b>
Glycolysis	2	2	0
Intermediate step	0	2	
Krebs cycle	2	6	2
<b>Total</b>	<b>4</b>	<b>10</b>	<b>2</b>

- ATP produced from complete oxidation of 1 glucose using aerobic respiration

Pathway	By substrate-level phosphorylation	By oxidative phosphorylation	
		From NADH	From FADH
Glycolysis	2	6	0
Intermediate step	0	6	
Krebs cycle	2	18	4
<b>Total</b>	<b>4</b>	<b>30</b>	<b>4</b>

- 36 ATPs are produced in eukaryotes.

# Respiration

- Aerobic respiration: The final electron acceptor in the electron transport chain is molecular oxygen ( $O_2$ ).
- Anaerobic respiration: The final electron acceptor in the electron transport chain is not  $O_2$ . Yields less energy than aerobic respiration because only part of the Krebs cycles operations under anaerobic conditions.

**Table 5.4 Comparison of Aerobic Respiration, Anaerobic Respiration, and Fermentation**

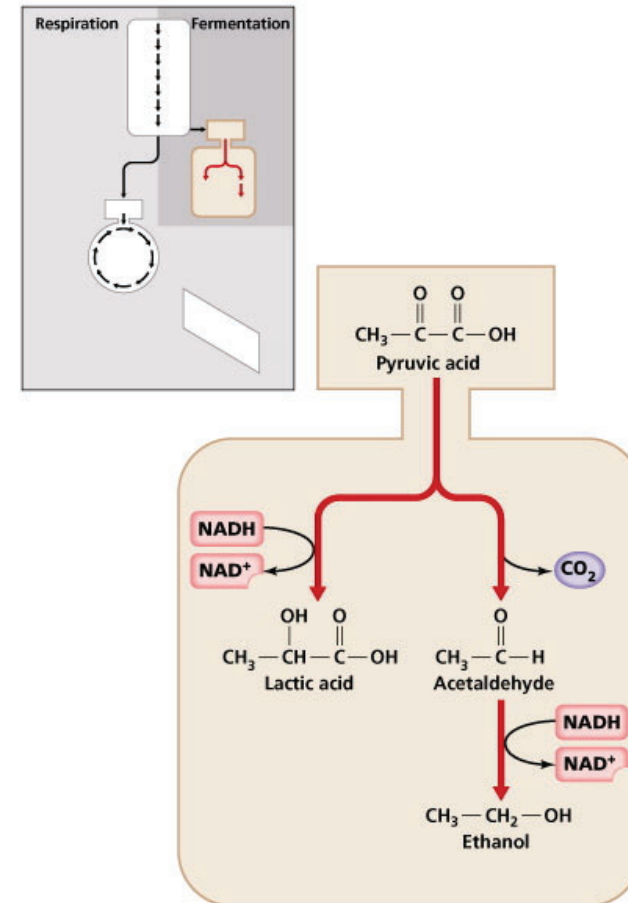
	Aerobic Respiration	Anaerobic Respiration	Fermentation
Oxygen required	Yes	No	No
Type of phosphorylation	Substrate-level and oxidative	Substrate-level and oxidative	Substrate-level
Final electron (hydrogen) acceptor	Oxygen	$NO_3^-$ , $SO_4^{2-}$ , or $CO_3^{2-}$	Organic molecules
Potential molecules of ATP produced	36–38	2–36	2

# Anaerobic Respiration

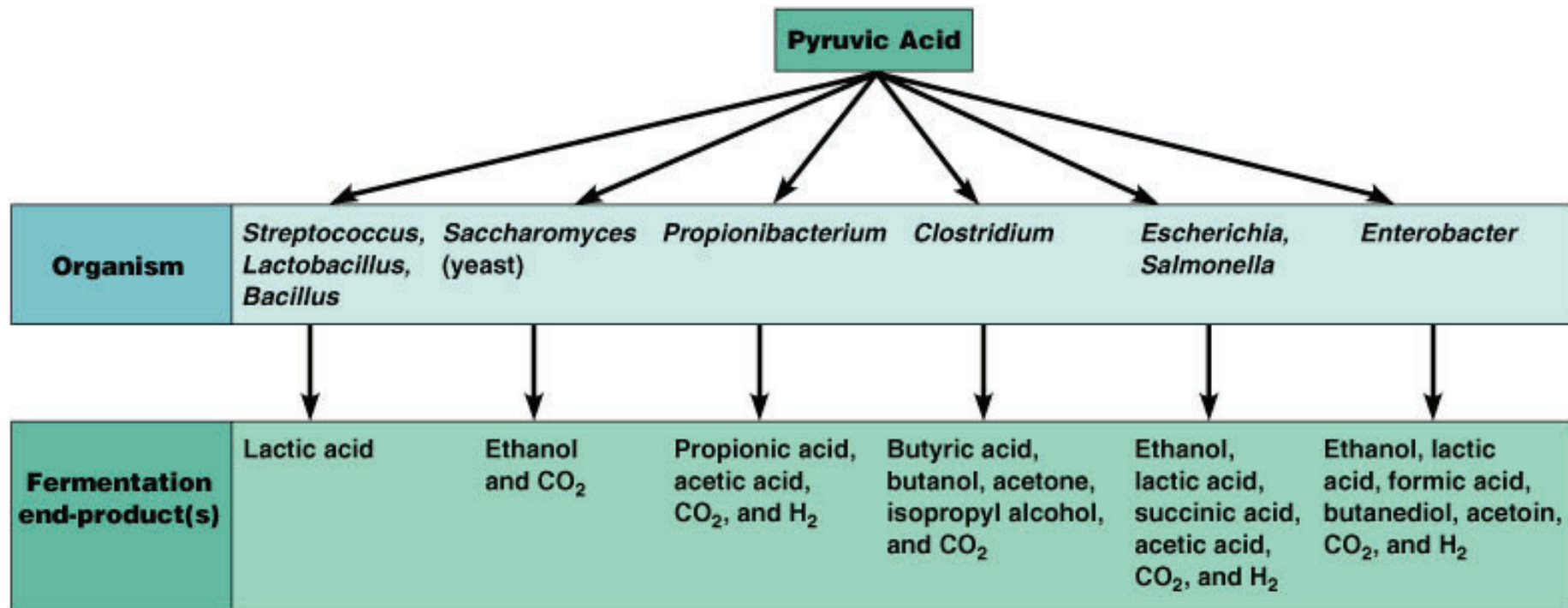
Electron acceptor	Products
$\text{NO}_3^-$	$\text{NO}_2^-$ , $\text{N}_2$ + $\text{H}_2\text{O}$
$\text{SO}_4^-$	$\text{H}_2\text{S}$ + $\text{H}_2\text{O}$
$\text{CO}_3^{2-}$	$\text{CH}_4$ + $\text{H}_2\text{O}$

# Fermentation

- Releases energy from oxidation of organic molecules
- Does not require oxygen
- Does not use the Krebs cycle or ETC
- Uses an organic molecule as the final electron acceptor

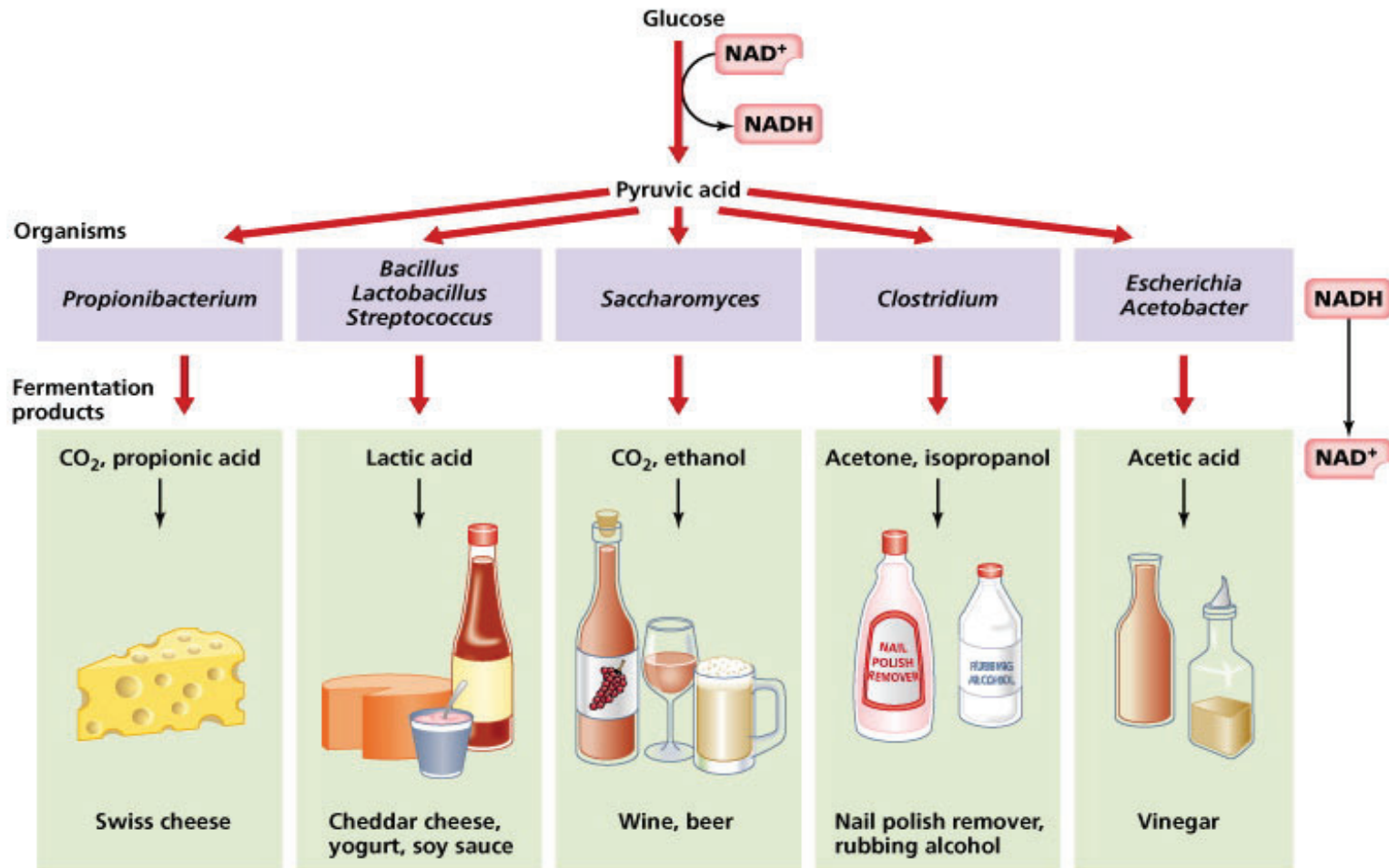


# Fermentation



(b)

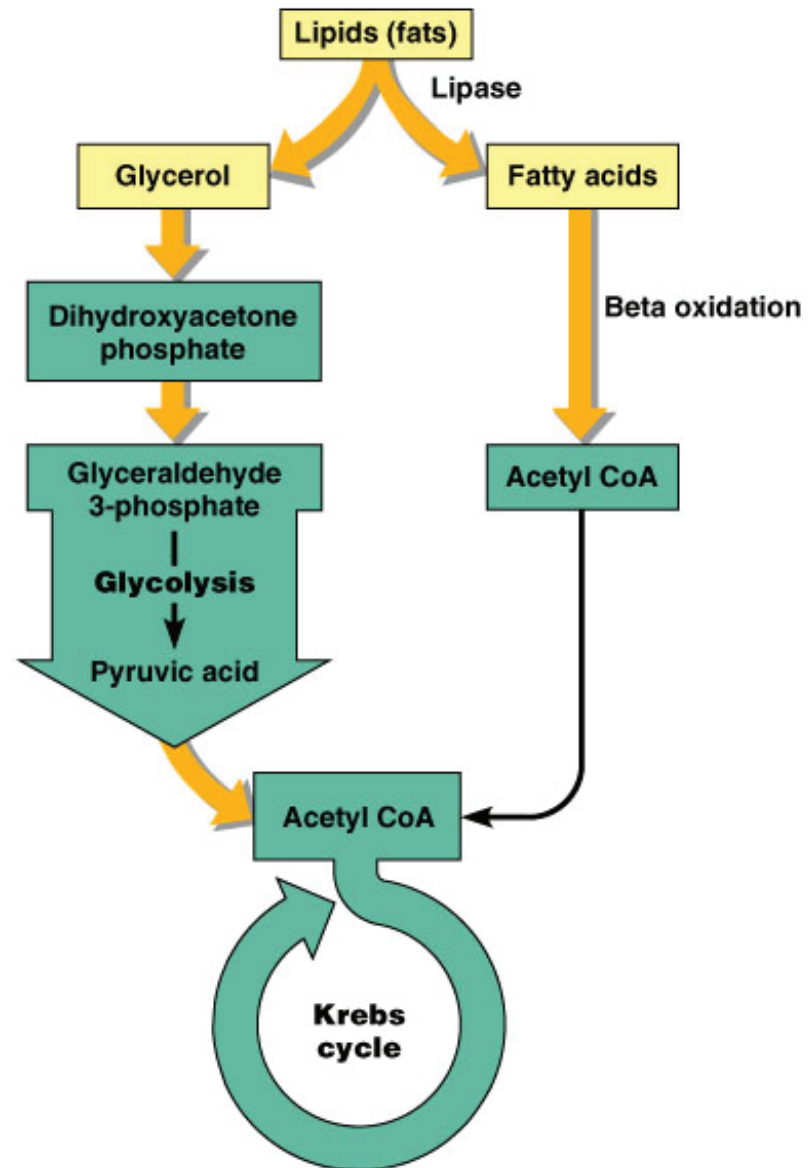
# Fermentation Products



# Fermentation

- **Alcohol fermentation** Produces ethyl alcohol + CO<sub>2</sub>
- **Lactic acid fermentation** Produces lactic acid.
  - **Homolactic fermentation** Produces lactic acid only.
  - **Heterolactic fermentation** Produces lactic acid and other compounds.

# Lipid Catabolism

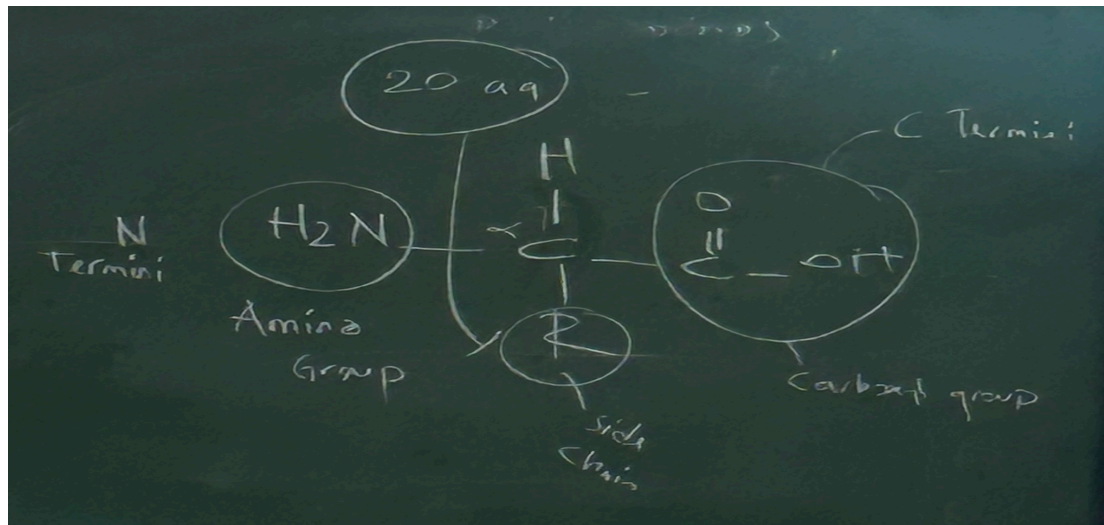


# Protein Catabolism

Vitamin B<sub>2</sub>  
Ubiquinone - non-protein carrier  
cytochromes iron containing protein Heme

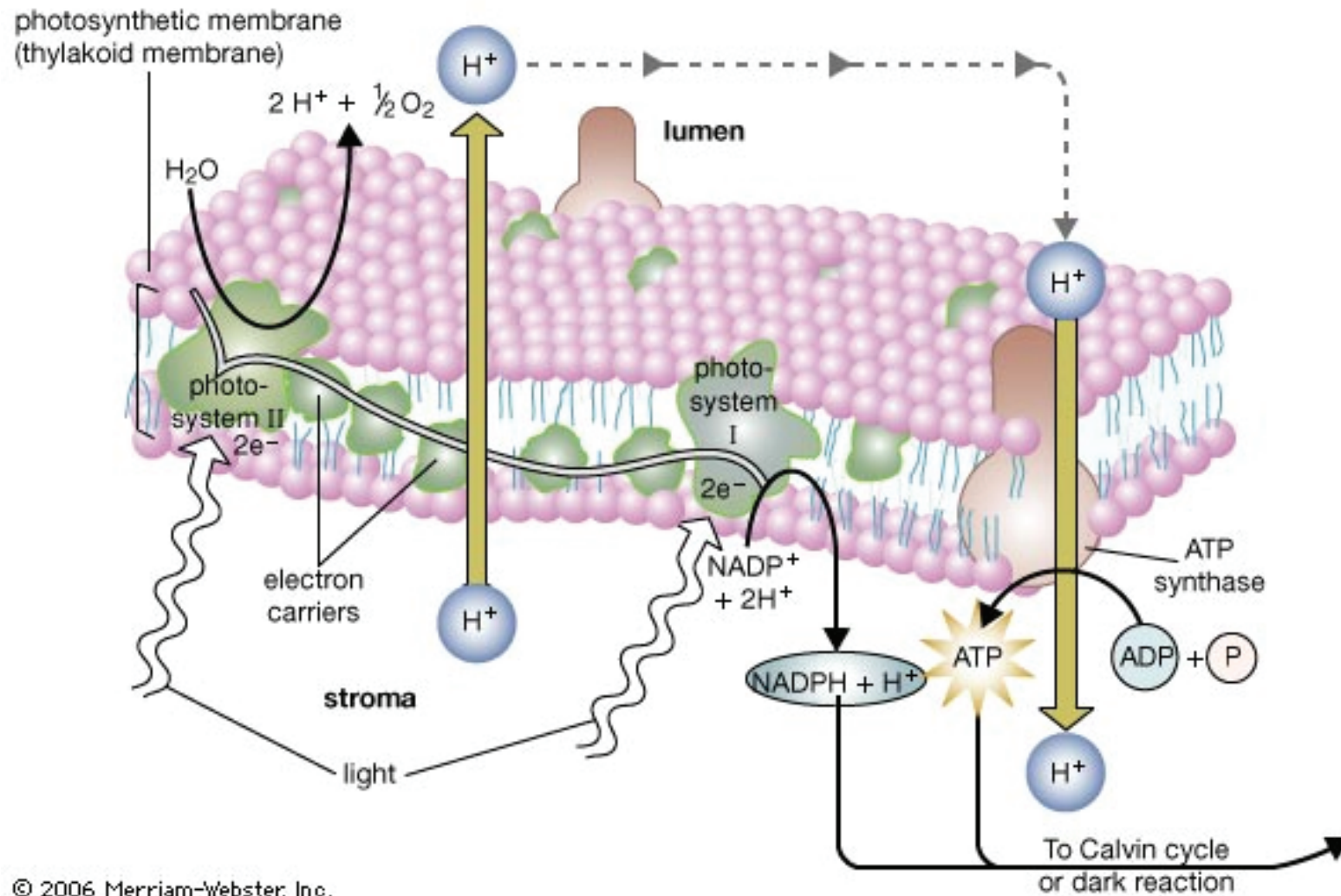
Protein  $\xrightarrow{\text{Extracellular proteases}}$  Amino acids

Deamination, decarboxylation, dehydrogenation  
 $\xrightarrow{\hspace{10em}}$  Organic acid  $\longrightarrow$  Krebs cycle



# Photosynthesis

- Photo: Conversion of light energy into chemical energy (ATP)
  - Light-dependent (light) reactions
- Synthesis: Fixing carbon into organic molecules
  - Light-independent (dark) reaction, Calvin-Benson cycle
- Oxygenic:  
$$6 \text{CO}_2 + 12 \text{H}_2\text{O} + \text{Light energy} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 + 6 \text{H}_2\text{O}$$
- Anoxygenic:  
$$\text{CO}_2 + 2 \text{H}_2\text{S} + \text{Light energy} \rightarrow [\text{CH}_2\text{O}] + 2 \text{A} + \text{H}_2\text{O}$$

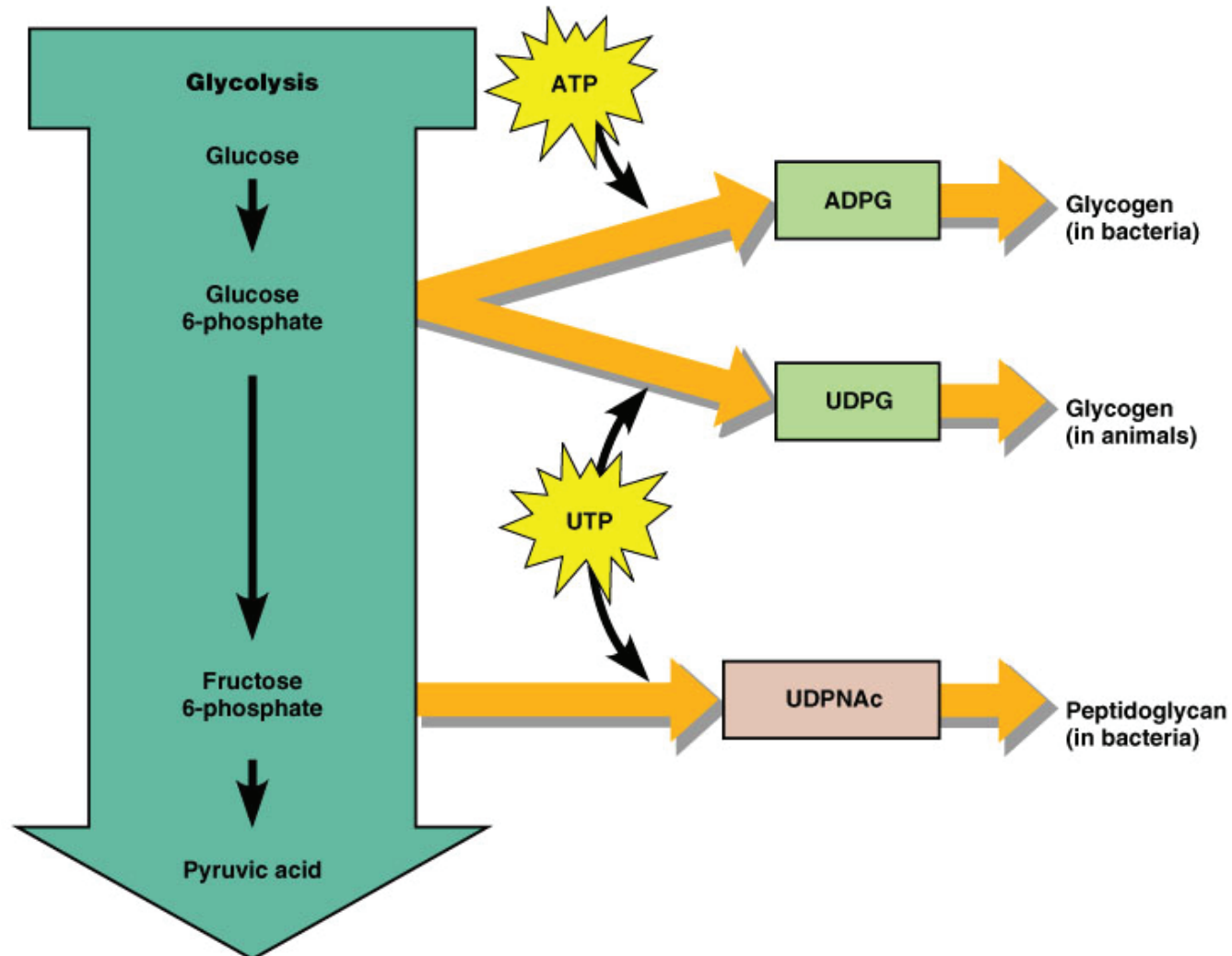


# Metabolic Diversity Among Organisms

Nutritional type	Energy source	Carbon source	Example
Photoautotroph	Light	CO <sub>2</sub>	Oxygenic: Cyanobacteria plants. Anoxygenic: Green, purple bacteria.
Photoheterotroph	Light	Organic compounds	Green, purple nonsulfur bacteria.
Chemoautotroph	Chemical	CO	Iron-oxidizing bacteria.
Chemoheterotroph	Chemical	Organic compounds	Fermentative bacteria. Animals, protozoa, fungi, bacteria.

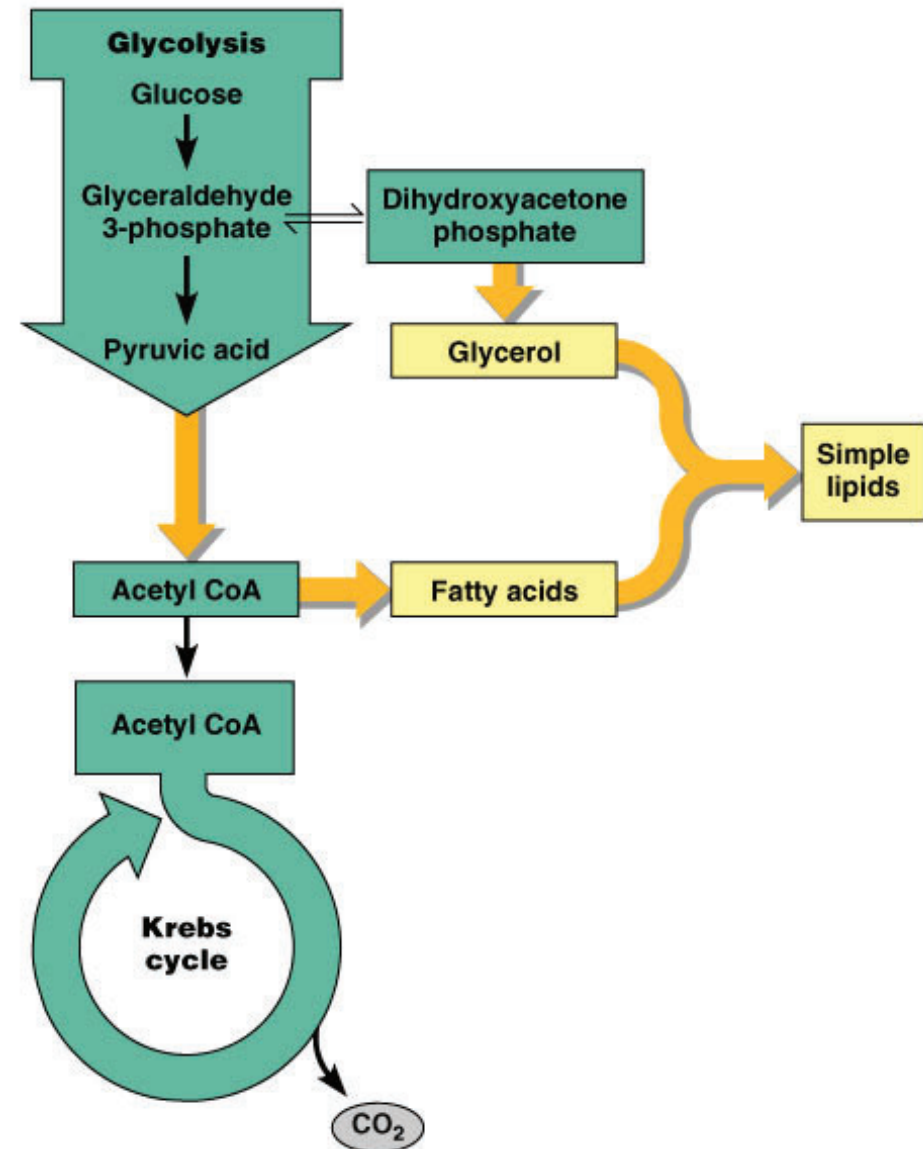
# Metabolic Pathways of Energy Use

- Polysaccharide Biosynthesis



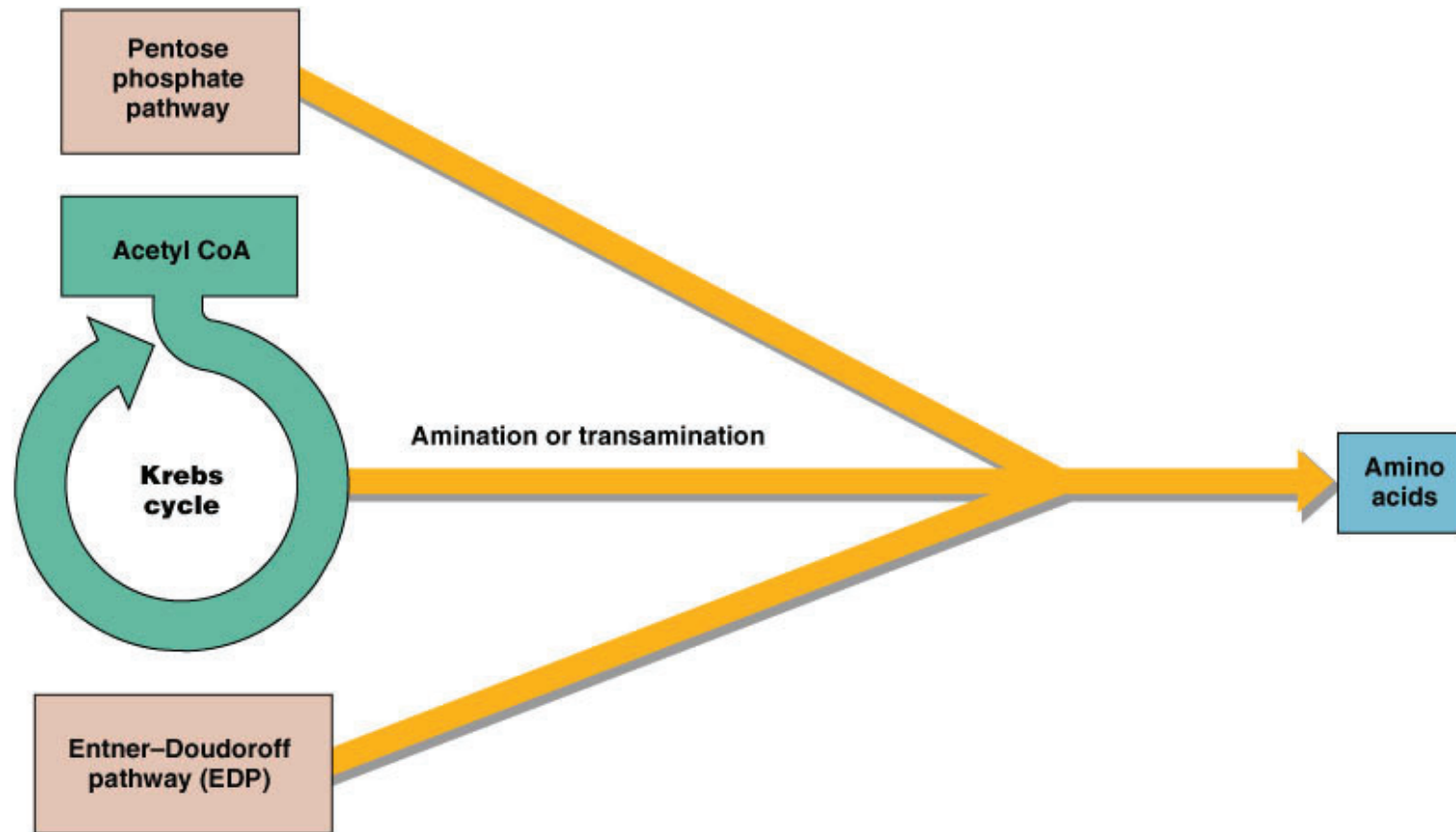
# Metabolic Pathways of Energy Use

- Lipid Biosynthesis



# Metabolic Pathways of Energy Use

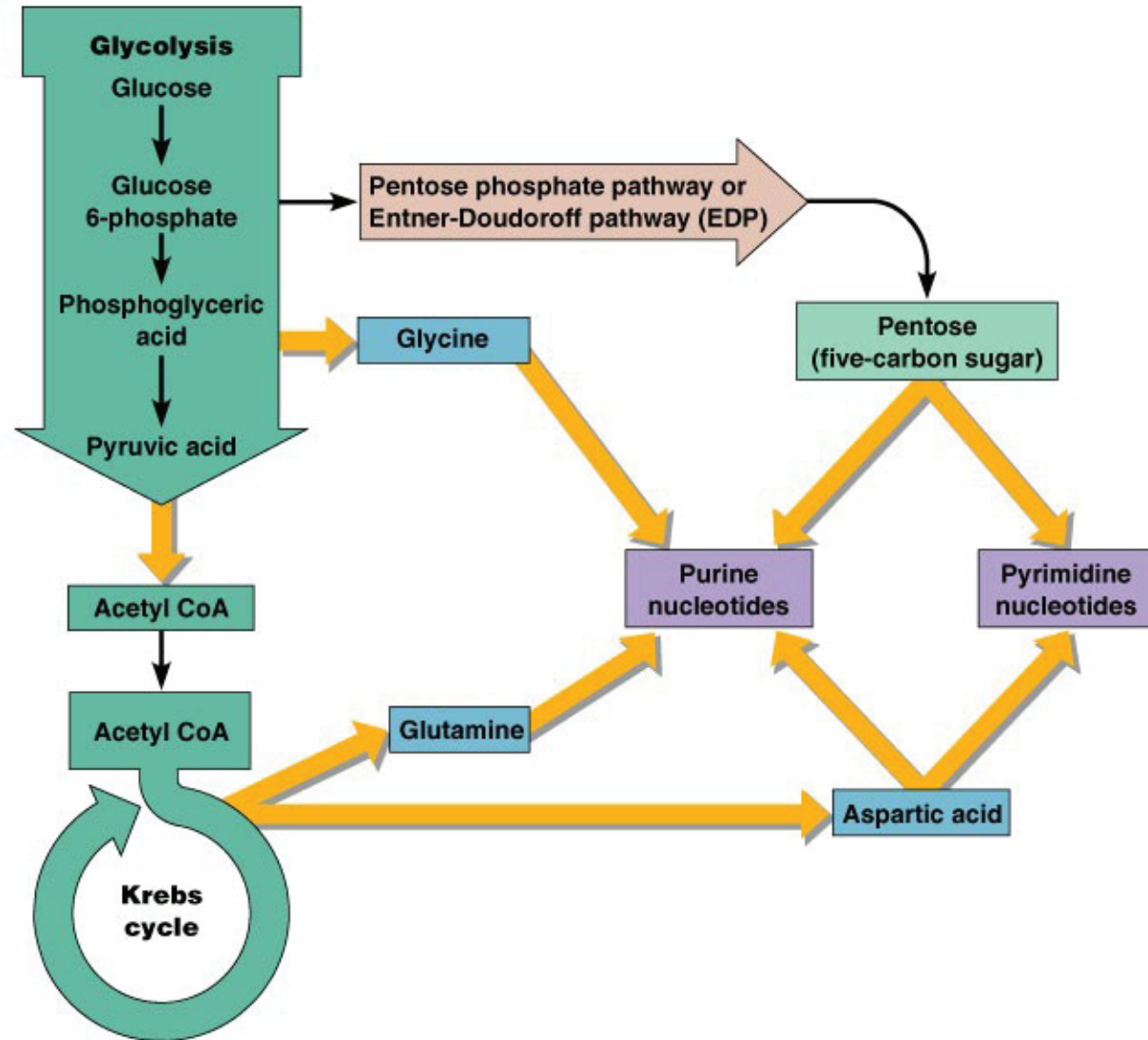
- Amino Acid and Protein Biosynthesis



**(a) Amino acid biosynthesis**

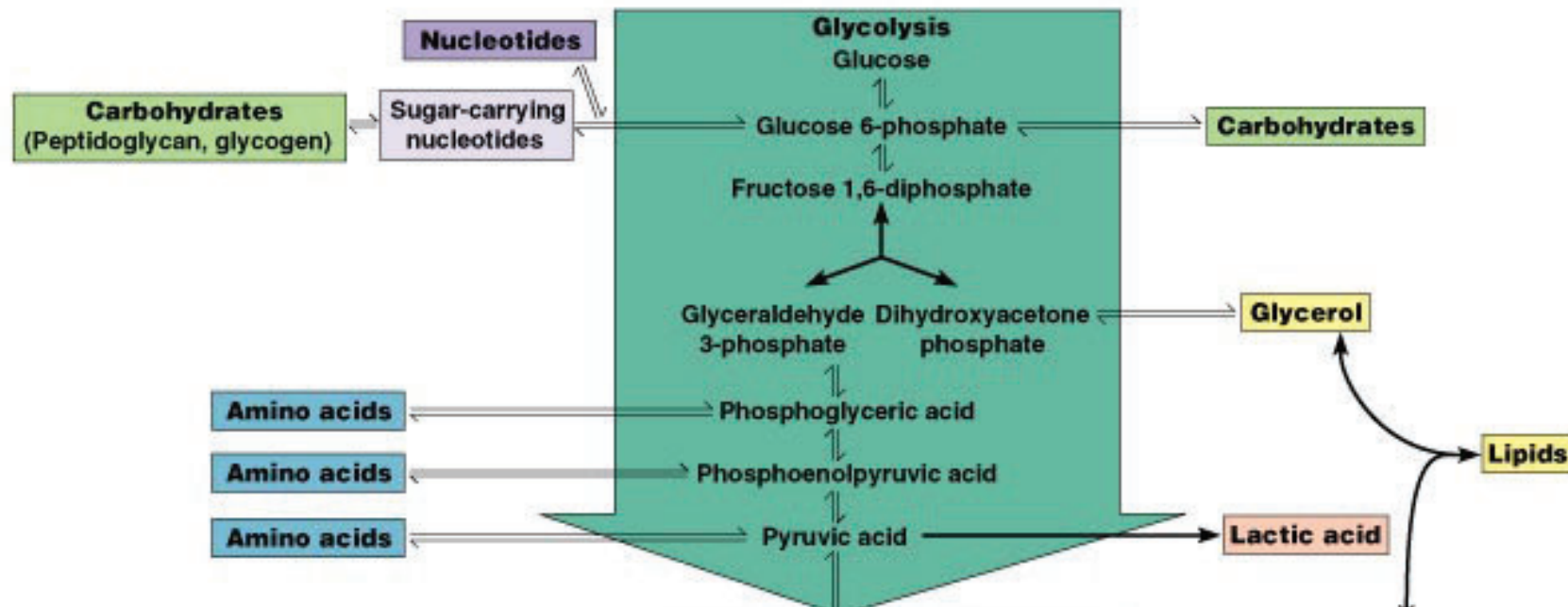
# Metabolic Pathways of Energy Use

- Purine and Pyrimidine Biosynthesis

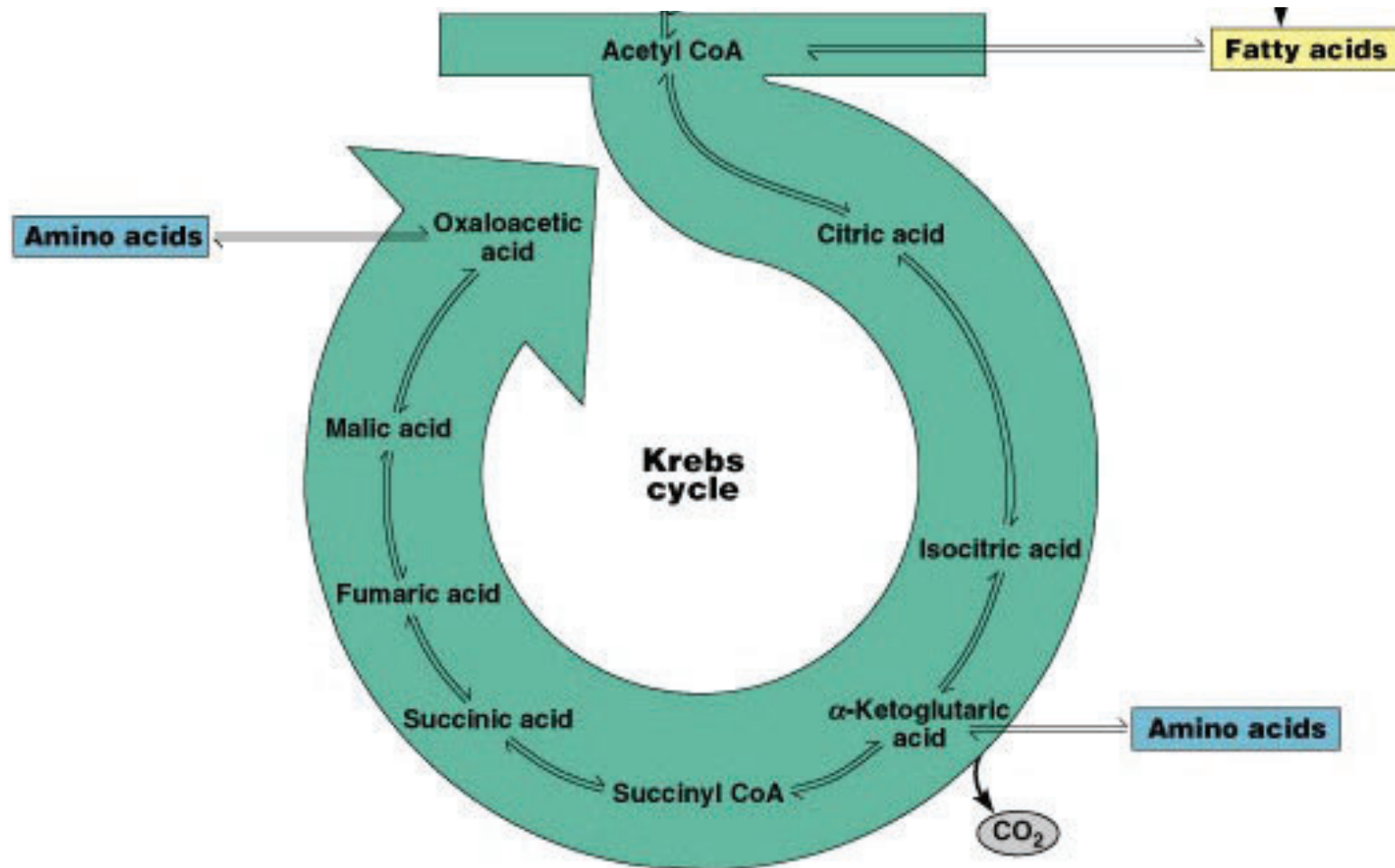


# Amphibolic Pathways

- Are metabolic pathways that have both catabolic and anabolic functions.



# Amphibolic Pathways



# In summary

