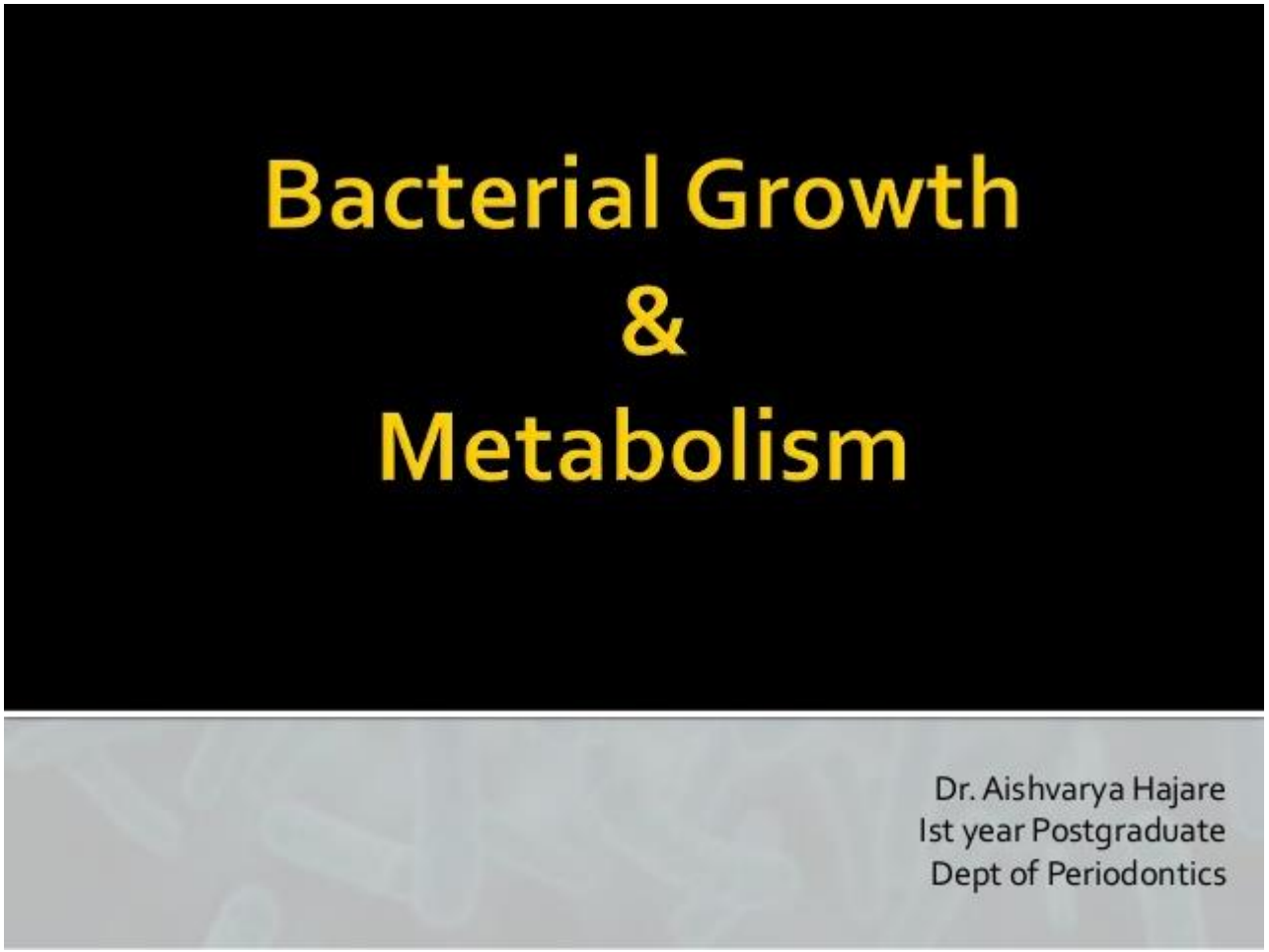

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- Bacterial growth
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- Bacterial flora in oral cavity
- A. actinomycetocomitans and P. gingivalis
- Conclusion
- References

- Eukaryote, any cell or organism that possesses a clearly defined nucleus.
- Has a nuclear membrane that surrounds the nucleus, in which the well-defined chromosomes are located.
- also contain organelles, including mitochondria, Golgi apparatus, endoplasmic reticulum and lysosomes.

- *"Encyclopædia Britannica"*

- Prokaryote is any organism that lacks a distinct nucleus and other organelles due to the absence of internal membranes.
- The cell membrane is made up of phospholipids and constitutes the cell's primary osmotic barrier.
- The cytoplasm contains ribosomes, which carry out protein synthesis, and a double-stranded deoxyribonucleic acid (DNA) chromosome, which is usually circular.

- *"Encyclopædia Britannica"*

Prokaryotic vs Eukaryotic Cells

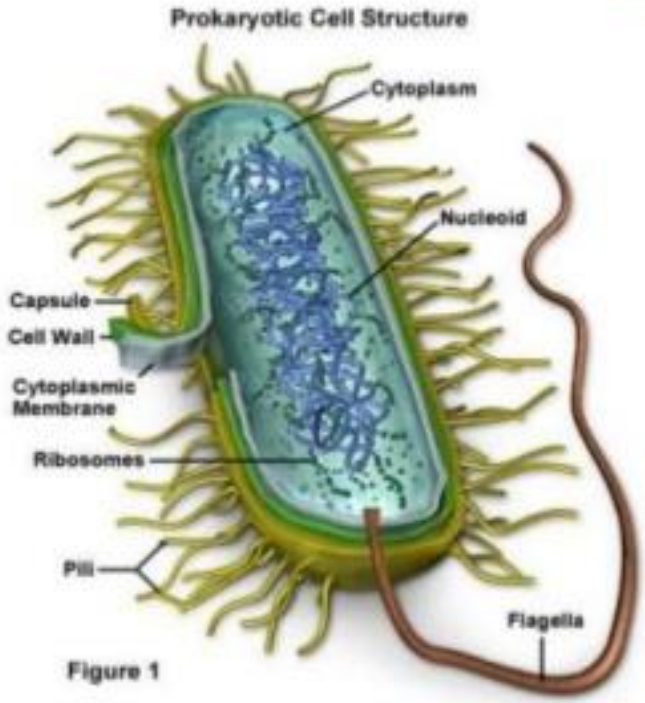
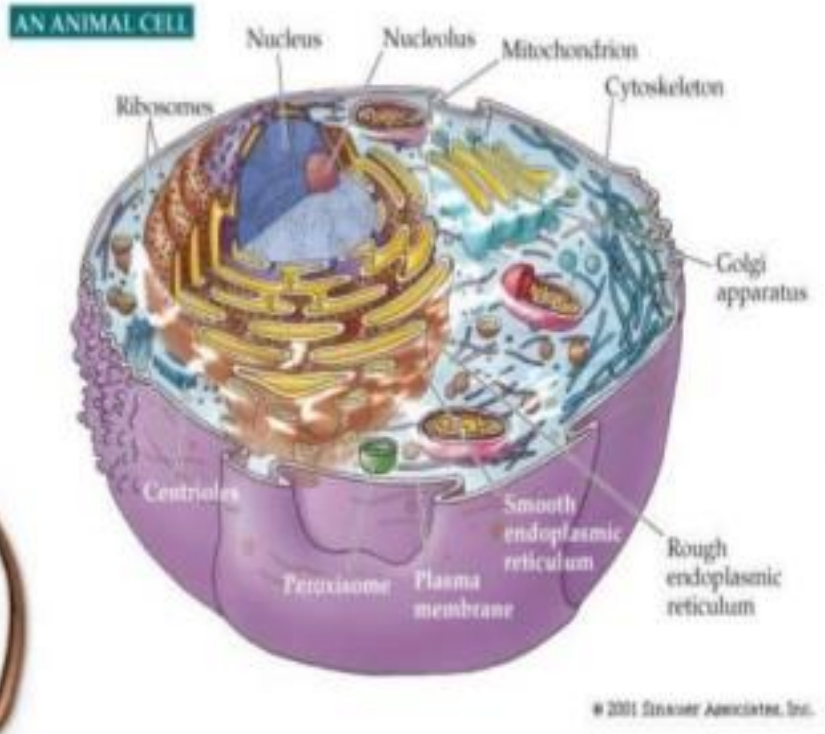


Figure 1



Classifying Microbes According to Their Energy and Carbon Sources.

- **Based on energy source**
  - **Phototrophs**
    - Use light as an energy source; photosynthesize.
  - **Chemotrophs**
    - Use inorganic and organic chemicals.
- **Based on carbon source**
  - **Autotrophs**
    - Use carbon dioxide.
  - **Heterotrophs**
    - Do not use carbon dioxide as their carbon source

Bacterial growth

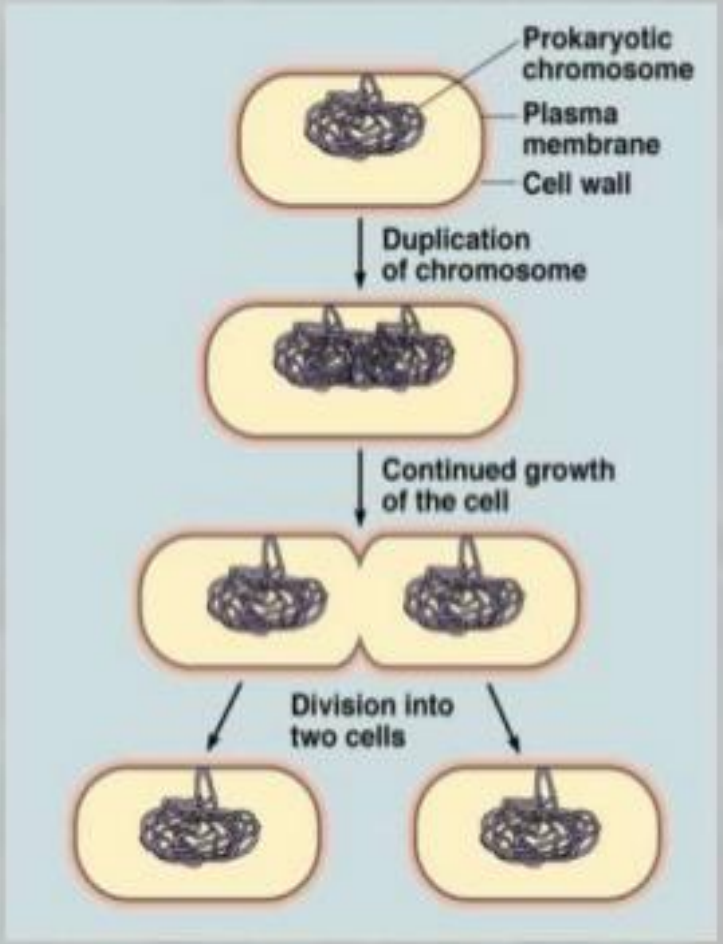
- Cells are the most fundamental units of life.
- All living organisms are made of one or more.
- Cells reproduce by copying their genetic material and then dividing—a parent cell giving rise to daughter cells.

- Types of Cell Division:
- **1) Binary Fission**
- **2) Mitosis & Meiosis**

- It refers to an increase in cell numbers, not in cell size.

Binary Fission

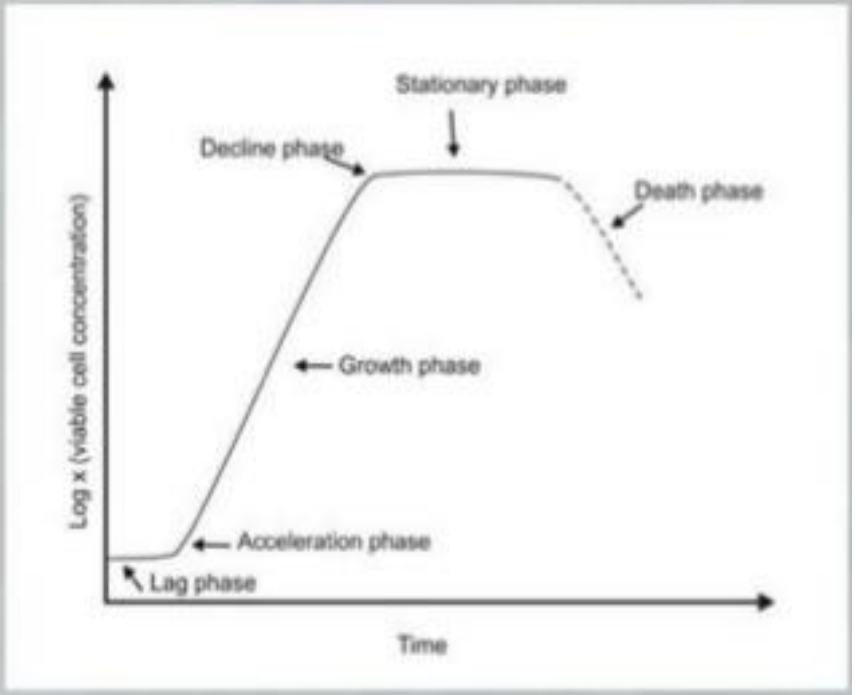
- The reproduction of prokaryotic cells (bacteria and bacteria-like Archaea) is accomplished through binary fission.
- A bacterial cell that is ready to divide first copies its genetic material, called the nucleoid—a single, circular chromosome of DNA (deoxyribonucleic acid).
- The two chromosomes, each attached to the plasma membrane, move apart as the cell elongates.

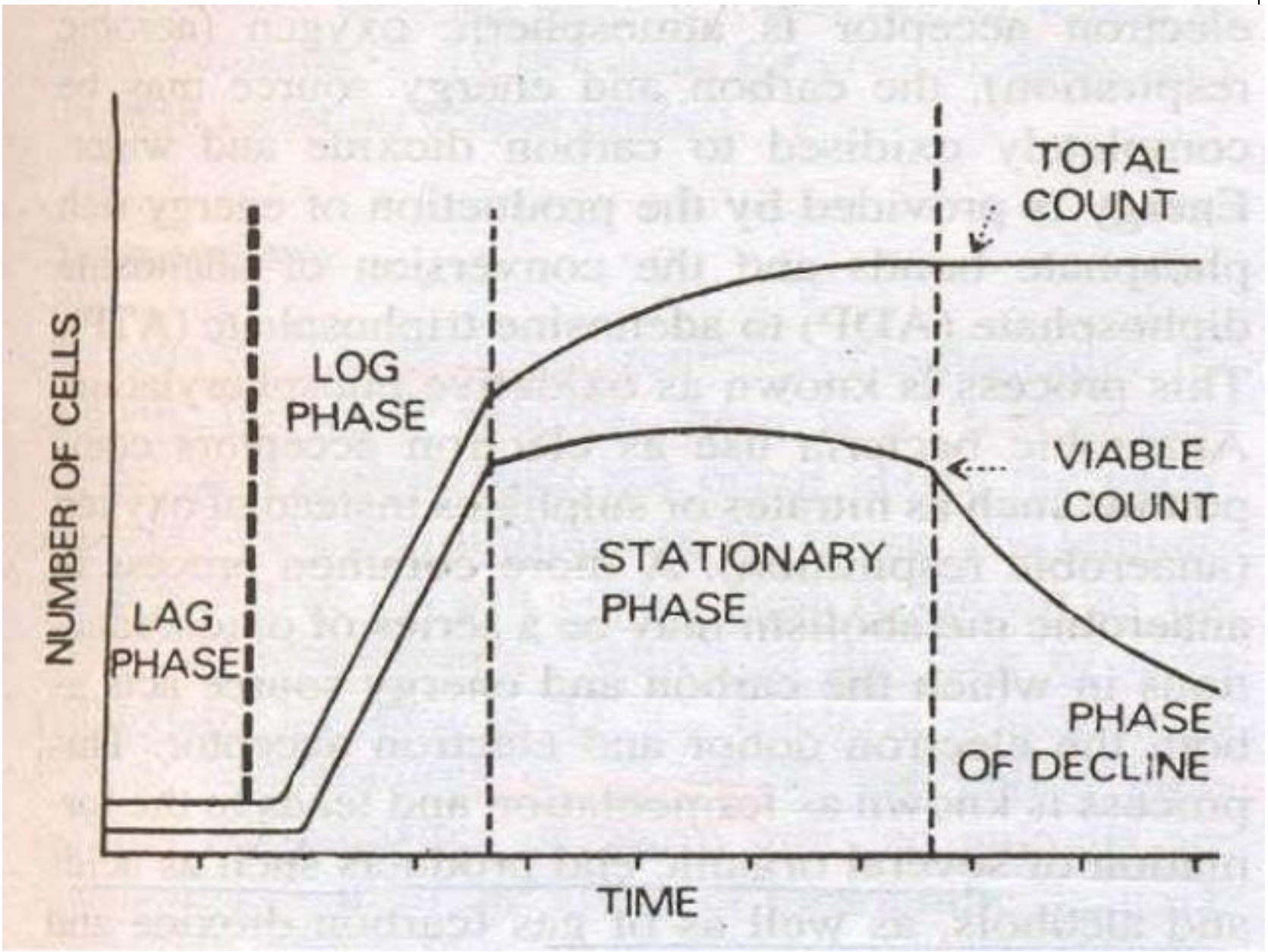


Bacterial growth curve

- Illustrates the dynamics of growth
Phases of growth

1. Lag phase
2. Exponential or logarithmic (log) phase
3.
4. Stationary phase
5. Death phase (decline phase)




TOTAL
COUNT
LOG
PHASE
VIABLE
COUNT
STATIONARY
PHASE
LAG
PHASE
PHASE
OF DECLINE
NUMBER OF CELLS
TIME

LAG PHASE

- During this phase, bacteria are growing in size, but they are not undergoing binary fission.
- Hence, there is no increase in cell number.
- The bacteria are adapting to the new environment and are synthesizing cellular components such as ribosomes, enzymes, and other proteins.
- Bacteria have the maximum cell size towards the end of the lag phase.

LOG PHASE

- This phase is also referred to as the exponential phase because there is a logarithmic increase in cell number.
- This exponential growth is expressed as the bacteria's generation time.
- During this phase, the conditions are optimal for growth and binary fission occurs.
- In the log phase, cells are smaller and stain uniformly.

STATIONARY PHASE

- There is no net increase or decrease in cell number in this stage. In other words, cell growth (division) equals cell death.
- The birth rate decreases due to limited nutrients, lack of space, and the build up of secondary metabolic products (e.g. toxins).
- The insufficient supply of nutrients also causes some bacteria to form spores during this phase.
- Cells frequently are gram variable and show irregular staining due to the presence of intracellular storage granules.

PHASE OF DECLINE

- This phase is characterized by an exponential death of cells.
- When the media runs out of nutrients and there are too many toxins, cells begin to die at a faster rate.
- Involution forms are common in the phase of decline.

FACTORS AFFECTING GROWTH

1. Water
2. Oxygen
3. Carbon dioxide
4. Temperature
5. Hydrogen ion concentration
6. Light
7. Osmotic pressure
8. Symbiosis and antagonism

WATER

- Moisture is essential for the life of bacteria. Most processes taking place in a bacterial cell are in a water base.
- 80% of bacterial cell consists of water.
- Dehydration is detrimental for most bacteria eg. Treponema pallidum. But some like staphylococcus can resist drying for months.
- Spores are particularly resistant to dessication and may survive in the dry state for several decades.

CARBON DIOXIDE

- Approximately half of dry weight
- CO_2 is provided by cellular metabolism and from environment.
- Autotrophic organisms are able to use carbon dioxide as source of carbon.
- Heterotrophic bacteria require some amount of carbon dioxide from exogenous sources.
- 5-10 % CO_2 is supplied for them in culture.
- Capnophilic = requiring excess amount of CO_2 eg Brucella abortus (10% CO_2).

- The carbon available in the carbohydrate sugar molecules is cycled further by microorganisms in a series of reactions that tricarboxylic acid (or TCA) cycle.
- The breakdown of the carbohydrate serves to supply energy to the microorganism.
- This process is also known as respiration.
- In anaerobic environments, microorganisms can cycle the carbon compounds to yield energy in a process known as fermentation

TEMPERATURE

- PSYCHROPHILES = bacteria which grow below 20°C, e.g. soil and water saprophytes. Up to -7°C reported.
- MESOPHILES = bacteria which grow between 20-40°C. e.g.. Most pathogenic bacteria are mesophiles. Wide range e.g. Pseudomonas 5-43°C, narrow range e.g. Gonococcus 30-39°C.
- THERMOPHILES = bacteria which grow at higher temperature i.e. 60-80°C. e.g. Bacillus stearothermophilus. Up to 250°C reported.

- THERMAL DEATH POINT = The lowest temperature that kills a bacterium under standard conditions in a given time.
- Under moist conditions most vegetative, mesophilic bacteria have a thermal death point 50 to 65°C and most spores between 100 and 120°C

HYDROGEN ION CONCENTRATION

- The pH requirement of bacteria is also variable.
- Most bacteria have an average pH requirement of 7.2-7.6 which matches with pH in human body environment.
- Some bacteria grow in acidic pH. Eg lactobacilli in pH=3.
- Some bacteria grow in alkaline pH. Eg. Alkaligenes at pH = 10.5

LIGHT

- Most bacteria prefer darkness for growth.
- Cultures die if exposed to sunlight.
- However some bacteria require sunlight and are called phototropic.
- Exposure to light may influence pigment production.
- Photochromogenic mycobacteria form a pigment only on exposure to light and not when incubated in the dark

OSMOTIC PRESSURE

- There is a wide range of osmotic tolerance found in bacteria.
- 0.5% NaCl is added in culture media to provide suitable osmolarity.
- PLASMOLYSIS = Sudden exposure to hypertonic solutions may cause osmotic withdrawal of water and shrinkage of protoplasm. This occurs more readily in gram negative than in gram positive bacteria.
- PLASMOPTYSIS = Sudden transfer from a concentrated solution to distilled water may cause plasmoptysis (excessive osmotic imbibition leading to swelling and rupture of the cell).

- Nitrogen: Found in all the amino acids, nitrogenous bases of nucleic acids, etc.
- Hydrogen: found in all biological molecules, Carbs, fats, proteins, nucleic acids, etc
- Phosphorous: found in nucleic acids, ATP, and phospholipdids of membranes
- Sulfur: found in 2 or 3 amino acids of microbes
- Trace elements: inorganic elements needed in very tiny concentrations (manganese, cobalt, Zn, Cr)
- s

- **Generation time** : The time required for a bacterium to give rise to two daughter cells under optimum conditions is known as generation time.
- **Kinetics of microbial growth** : unicellular organisms divide by binary fission each cell grows to full size, replicates its genetic material then divides into identical daughter cells , leading to exponential increase in cell numbers.

$1 \longrightarrow 2 \longrightarrow 4 \longrightarrow 8 \longrightarrow 2^n$

MAINTENANCE OF CELLS IN EXPONENTIAL PHASE (CONTINUOUS CULTURE)

- This is done by repeatedly transferring bacterial cells into fresh medium of identical composition.
- Transfer is done while they are multiplying in exponential phase.
- Two techniques are used:
  - Chemostat device
  - Turbidostat device
- Maintenance of bacteria in continuous culture is sometimes necessary in industrial and research purposes.

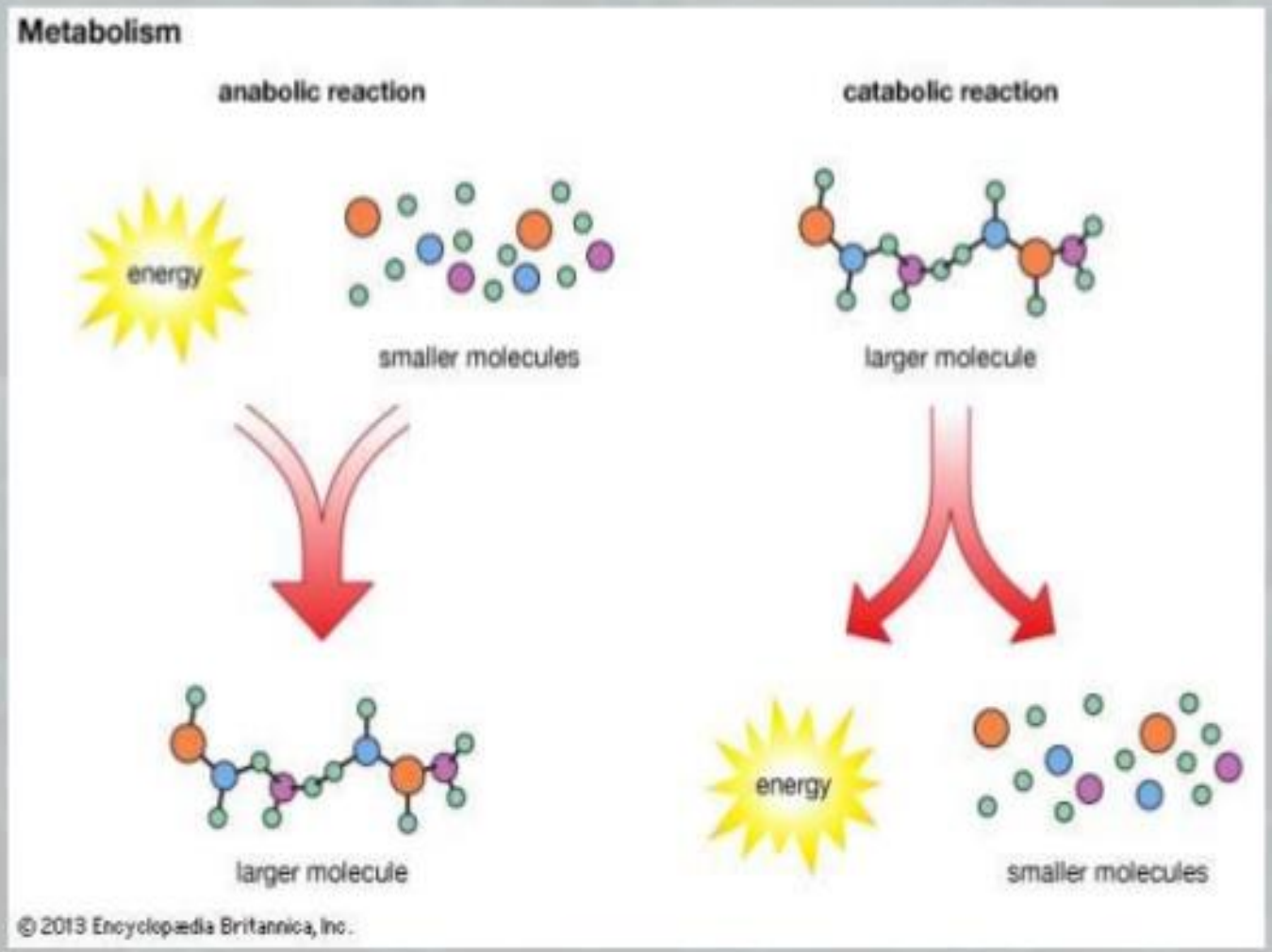
Bacterial growth in vivo

- In vitro and in vivo growth patterns of bacteria are different as the environmental conditions are different.
- In vivo growth of bacteria depends on our nutritional status, immunity, hormonal factors, pH, redox potential etc.
- There is a significant difference in the growth patterns of bacteria in human body and artificial medium.

BACTERIAL METABOLISM

- **METABOLISM** = the series of changes of a substance (carbohydrate, protein, fat) that take place within the bacterial cell from absorption to elimination is known as metabolism.
- **CATABOLISM** = breakdown of macromolecules into simpler micromolecules, absorption into cell, conversion into basic blocks including interconversion of ADP to ATP.
- **ANABOLISM** = a process by which the basic building blocks are utilized in synthesis of various cellular structures such as monomers and polymers.

- Aerobic bacteria obtain their energy and intermediates only through **OXIDATION** and energy is provided by ATP (oxidative phosphorylation).
- Anaerobic bacteria obtain their energy by **FERMENTATION** (substrate level phosphorylation).
- Facultative anaerobes may act in both ways.


Metabolism
anabolic reaction
catabolic reaction
energy
smaller molecules
larger molecule
larger molecule
energy
smaller molecules
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- Glucose is a key energy-storing molecule
- Nearly all cells metabolize glucose for energy
- Glucose metabolism is fairly simple
- Other organic molecules are converted to glucose for energy harvesting

Components of metabolism

Components	Functions
Enzymes	biological catalyst, facilitates each step of metabolic reaction by lowering the activation of energy reaction.
Adenosine triphosphate (ATP)	Serves as energy currency of cell
Energy source	Compound that is oxidized to release energy, also called as electron donor.
Electron carriers	Carry the electrons that are removed during oxidation of energy source.
Precursor metabolites	Intermediate metabolite that link anabolic & catabolic pathway.

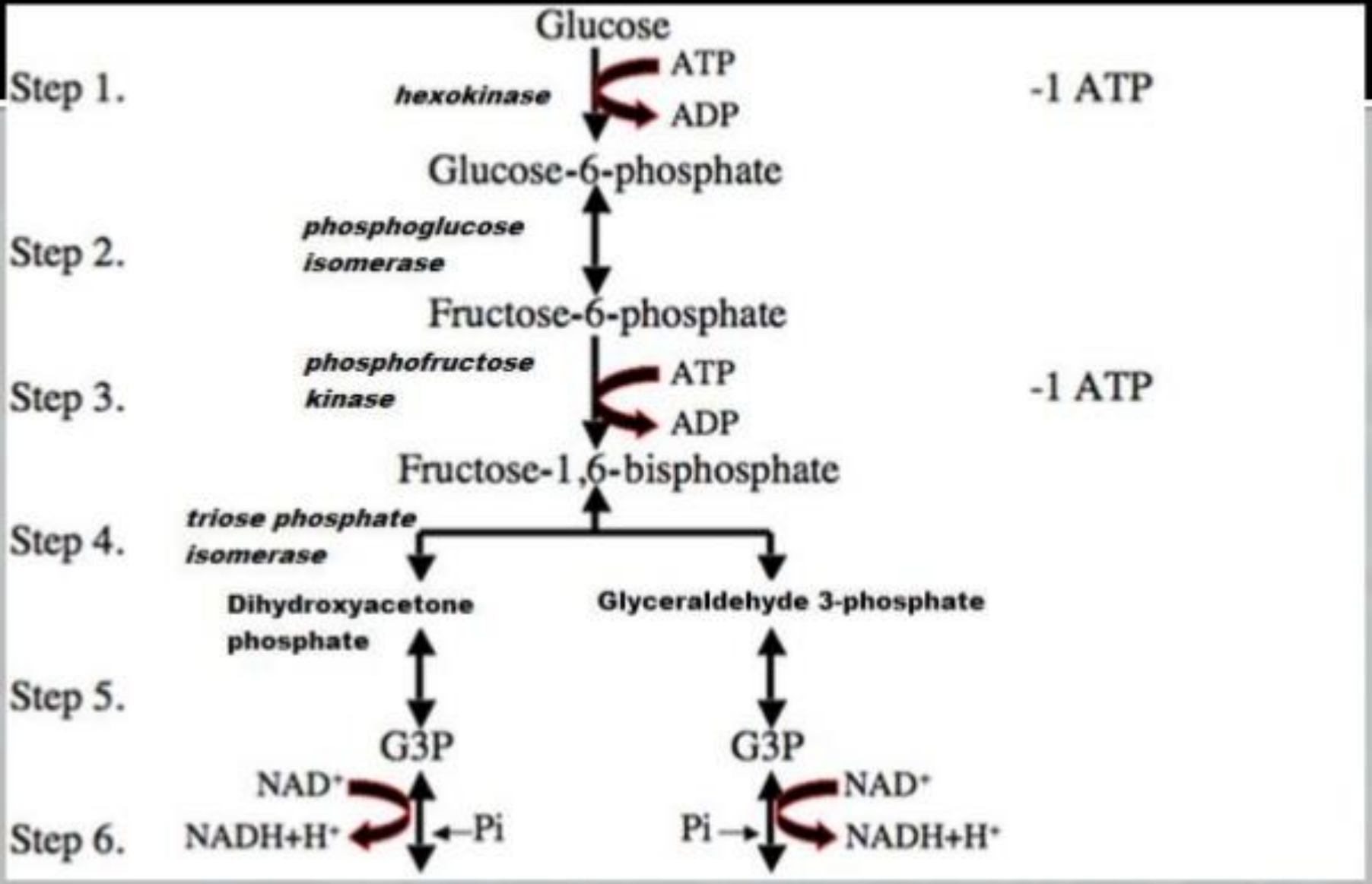
Overview of Glucose Breakdown

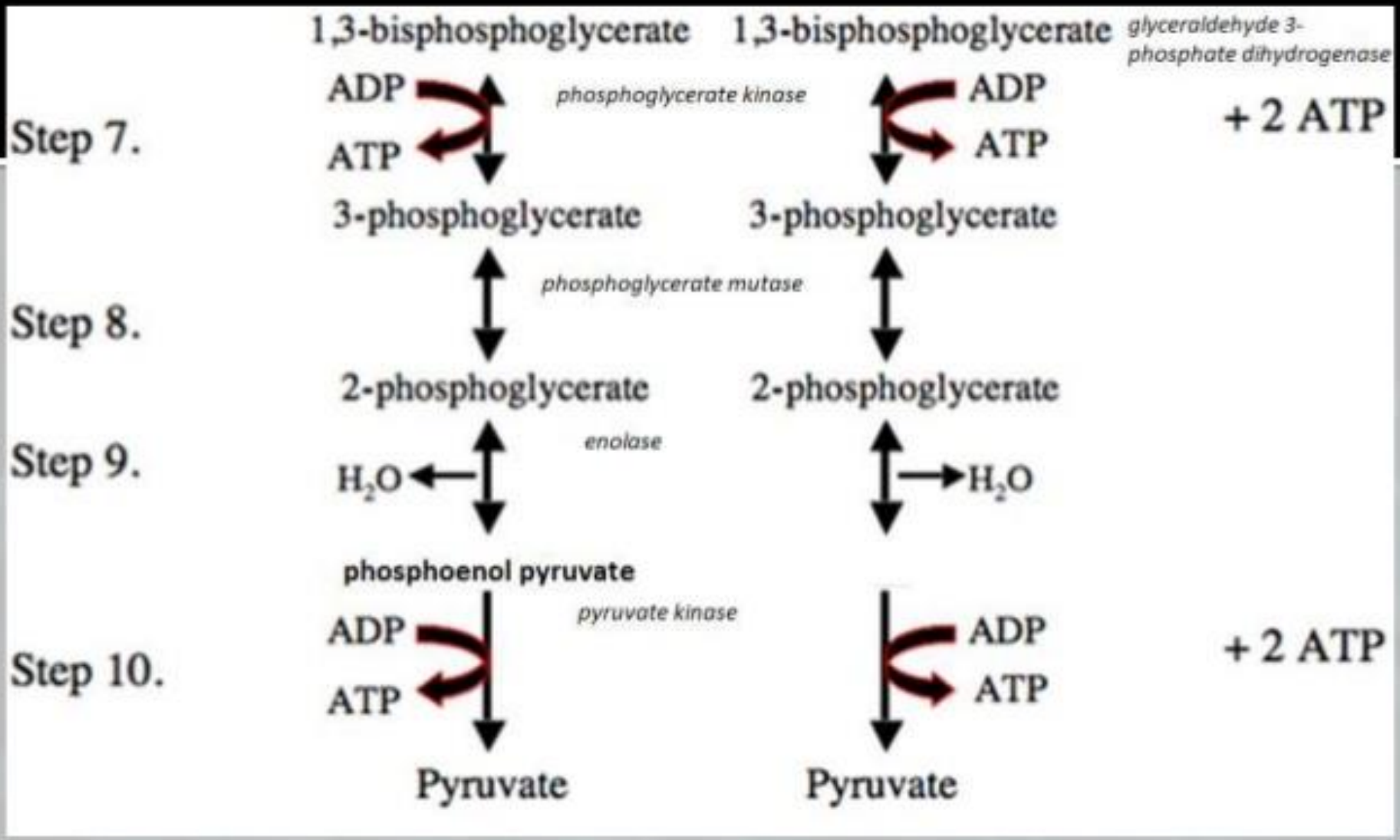
- The overall equation for the complete breakdown of glucose is:
- $C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + ATP$
- The main stages of glucose metabolism are:
  - **Glycolysis**
  - **Cellular respiration**

Overview of Glucose Breakdown - Glycolysis

- Glycolysis
- Occurs in the cytosol
- Does not require oxygen
- Breaks glucose into pyruvate
- Yields two molecules of ATP per molecule of glucose

- If oxygen is absent fermentation occurs
- **Pyruvate is converted into either lactate, or into ethanol and CO_2**
- If oxygen is present cellular respiration occurs


Glucose
Step 1.
hexokinase
ATP
ADP
-1 ATP
Glucose-6-phosphate
Step 2.
phosphoglucose isomerase
Fructose-6-phosphate
Step 3.
phosphofructose kinase
ATP
ADP
-1 ATP
Fructose-1,6-bisphosphate
Step 4.
triose phosphate isomerase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Step 5.
G3P
G3P
Step 6.
NAD+
NADH+H+
Pi
Pi
NAD+
NADH+H+


1,3-bisphosphoglycerate
1,3-bisphosphoglycerate
glyceraldehyde 3-phosphate dihydrogenase
Step 7.
ADP
ATP
phosphoglycerate kinase
ADP
ATP
+ 2 ATP
3-phosphoglycerate
3-phosphoglycerate
Step 8.
phosphoglycerate mutase
2-phosphoglycerate
2-phosphoglycerate
Step 9.
enolase
H_2O
H_2O
phosphoenol pyruvate
Step 10.
ADP
ATP
pyruvate kinase
ADP
ATP
+ 2 ATP
Pyruvate
Pyruvate

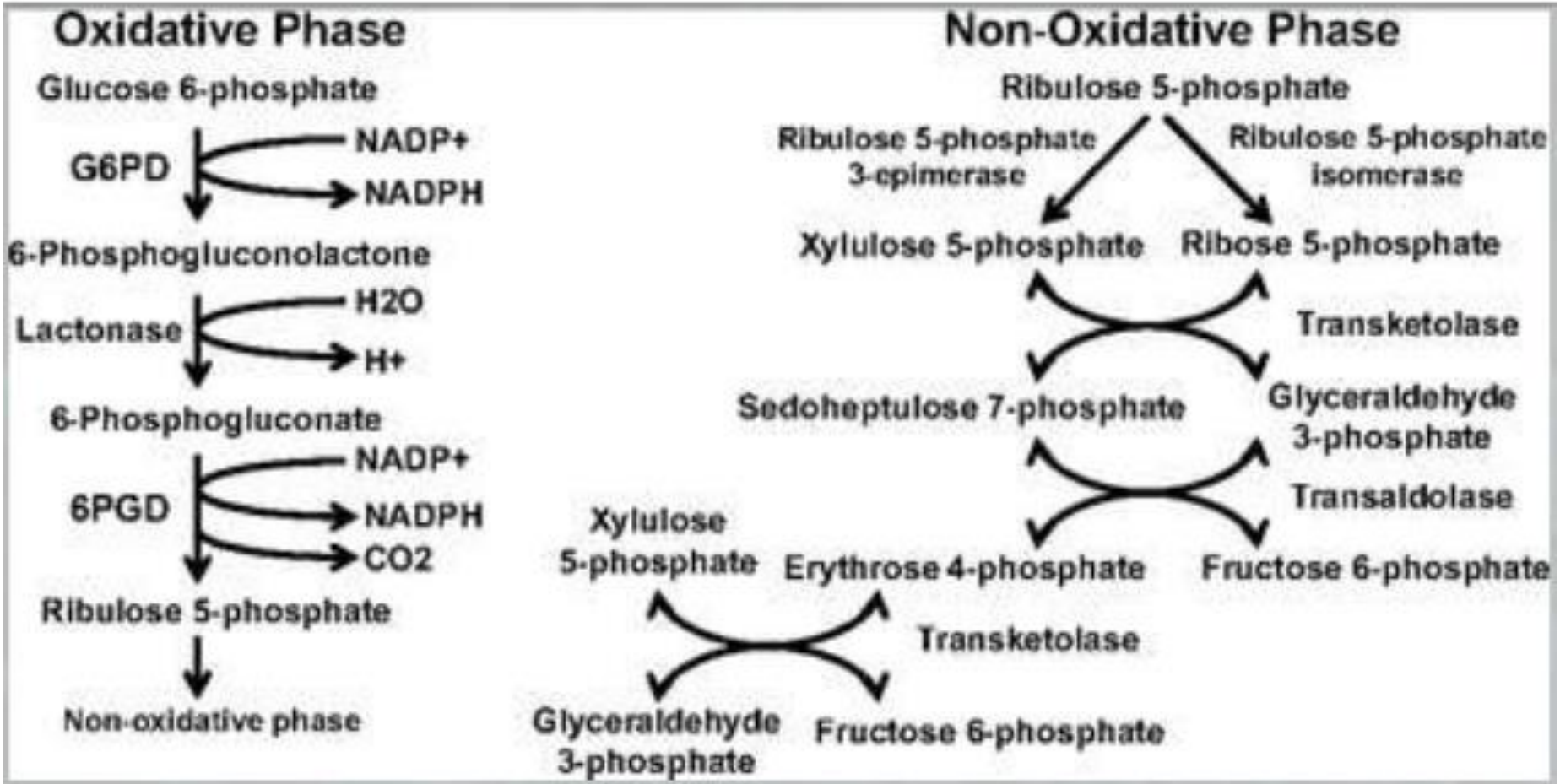
Pathways alternative to glycolysis

- Many bacteria have another pathway in addition to glycolysis for degradation of glucose.
- **1. Pentose Phosphate Pathway**
- **2. Entner Doudoroff Pathway**

Pentose phosphate pathway

- Hexose monophosphate shunt
- Occurs simultaneously with glycolysis & provide breakdown of both pentose sugar and glucose.
- Intermediate pentoses are used for nucleic acid synthesis, aminoacid synthesis
- Important producer of reduced coenzyme i.e. NADPH used for biosynthetic reaction.

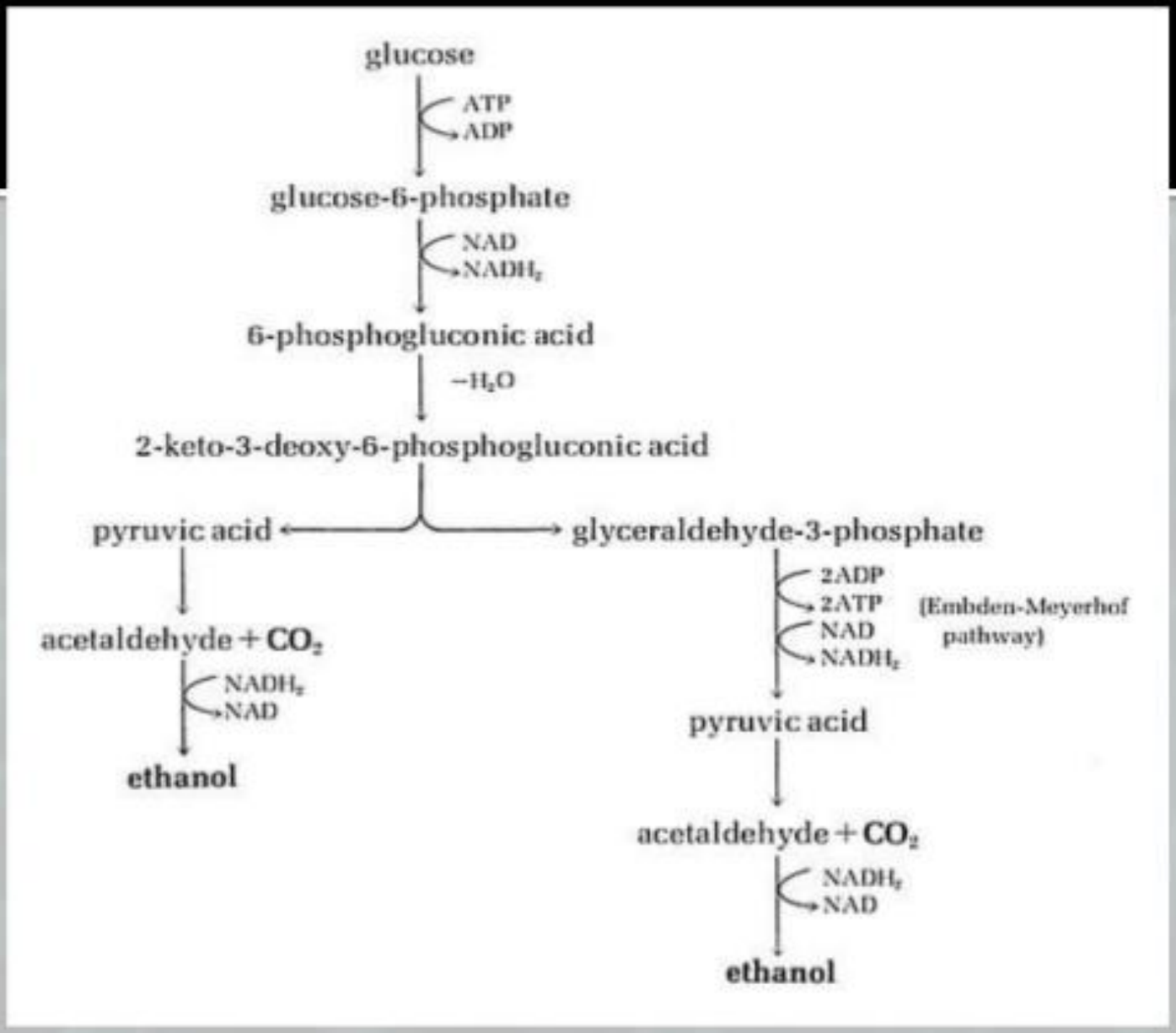
Stuart Hogg Essential microbiology 2nd edition John Wiley & Sons.Ltd 2005


Oxidative Phase
Glucose 6-phosphate
G6PD
NADP+
NADPH
6-Phosphogluconolactone
Lactonase
H2O
H+
6-Phosphogluconate
6PGD
NADP+
NADPH
CO2
Ribulose 5-phosphate
Non-oxidative phase
Non-Oxidative Phase
Ribulose 5-phosphate
Ribulose 5-phosphate 3-epimerase
Ribulose 5-phosphate isomerase
Xylulose 5-phosphate
Ribose 5-phosphate
Transketolase
Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
Transaldolase
Erythrose 4-phosphate
Fructose 6-phosphate
Xylulose 5-phosphate
Transketolase
Glyceraldehyde 3-phosphate
Fructose 6-phosphate

Entner-Doudoroff pathway

- Uses 6-phosphogluconate dehydratase and 2-keto-3-deoxyphosphogluconate aldolase to create pyruvate from glucose.
- Most of gram –ve bacteria like pseudomonas, rhizobium, agrobacterium.
- Produces 1 molecule NADH, 1 molecule NADPH and 1 molecule of ATP

Stuart Hogg Essential microbiology 2nd edition John Wiley & Sons.Ltd 2005


glucose
ATP
ADP
glucose-6-phosphate
NAD
NADH2
6-phosphogluconic acid
−H2O
2-keto-3-deoxy-6-phosphogluconic acid
pyruvic acid
glyceraldehyde-3-phosphate
2ADP
2ATP
NAD
NADH2
(Embden-Meyerhof pathway)
acetaldehyde + CO2
NADH2
NAD
ethanol
pyruvic acid
acetaldehyde + CO2
NADH2
NAD
ethanol

Overview of Glucose Breakdown- Cellular Respiration

- Cellular respiration - pyruvate obtained from glucose breakdown are channeled either to respiration or fermentation.
- Requires oxygen
- Breaks down pyruvate into carbon dioxide and water

Reactions in Mitochondrial Matrix (Eukaryotes) or Cytoplasm (Prokaryotes)

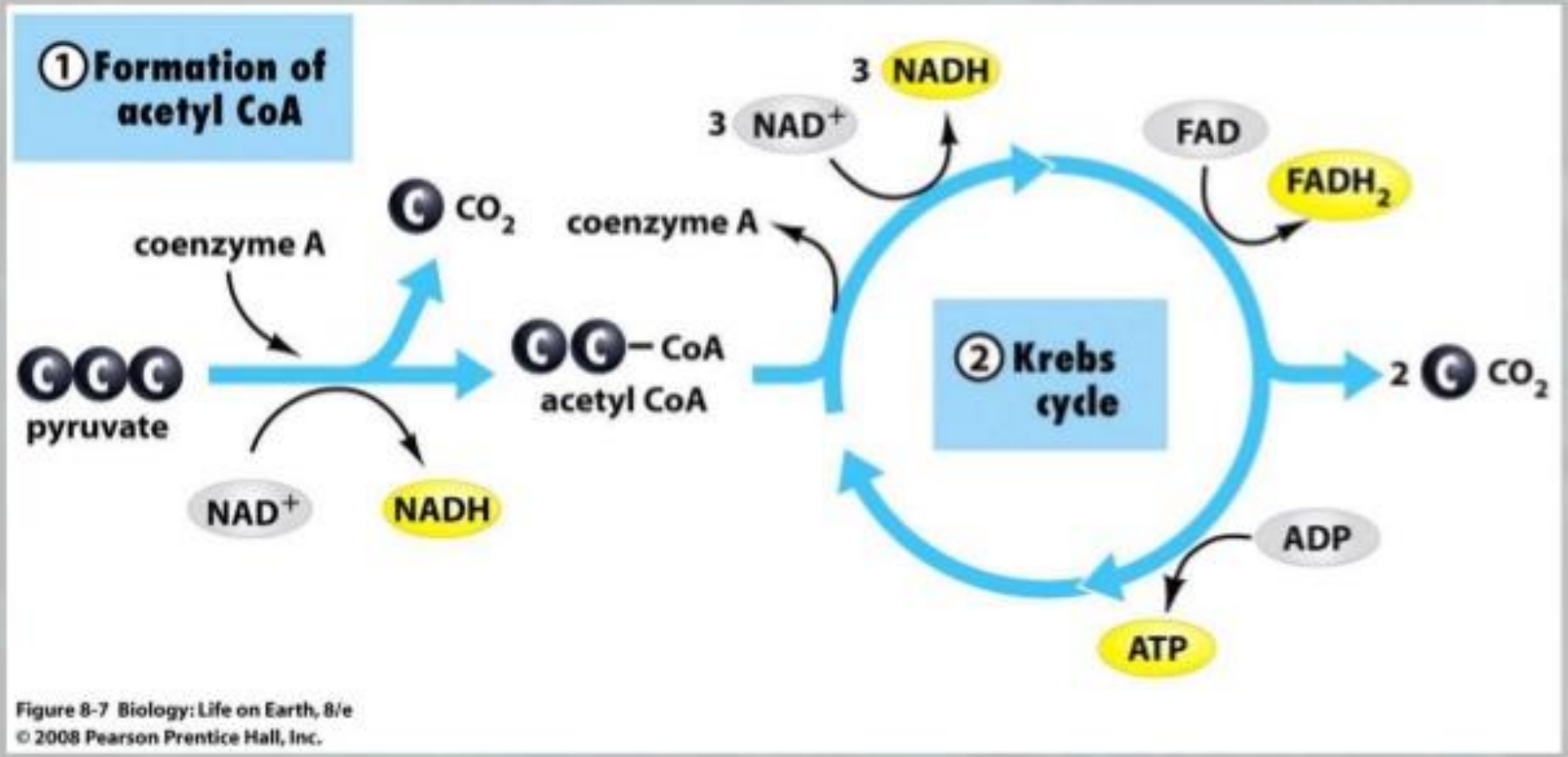
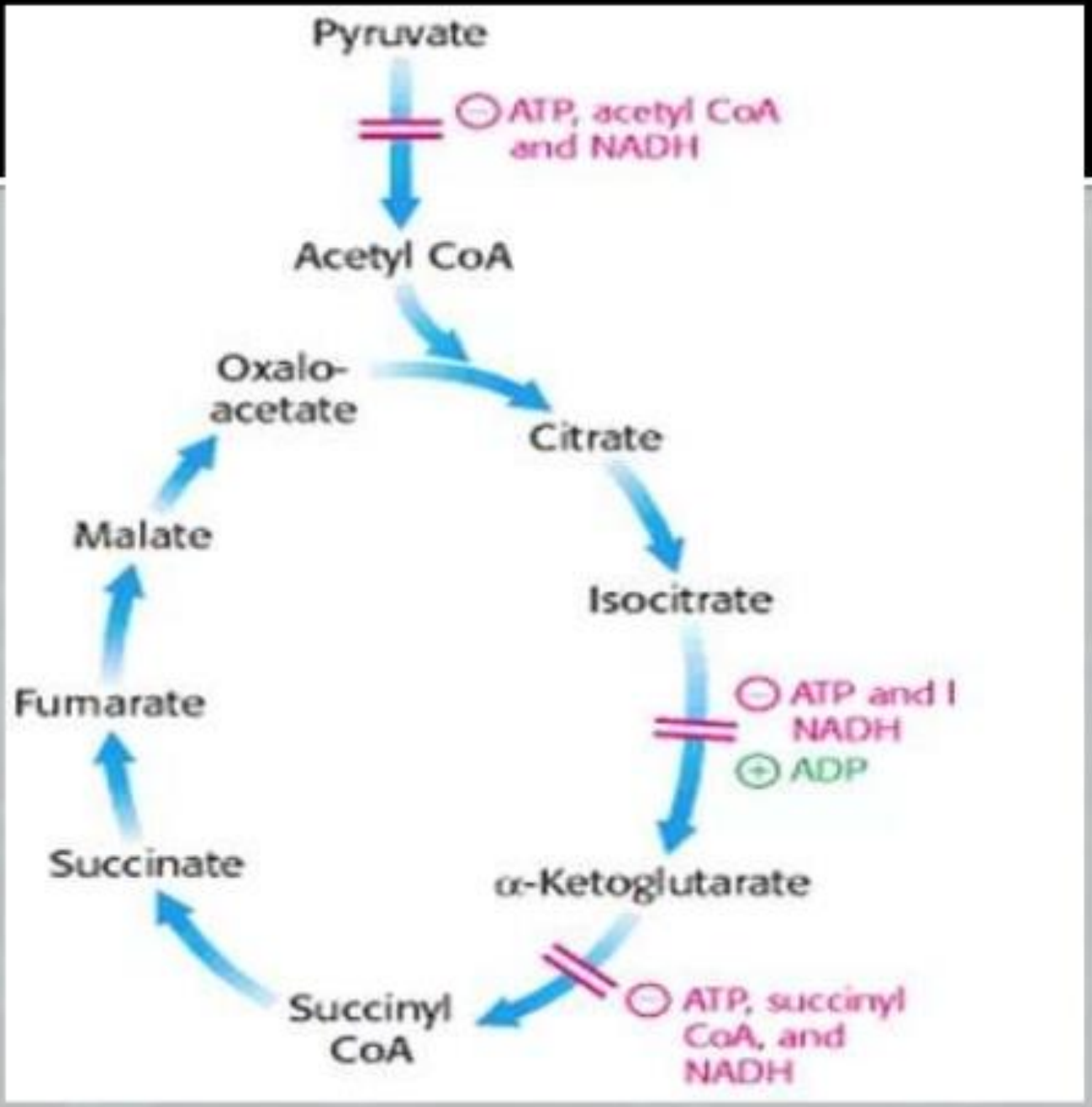


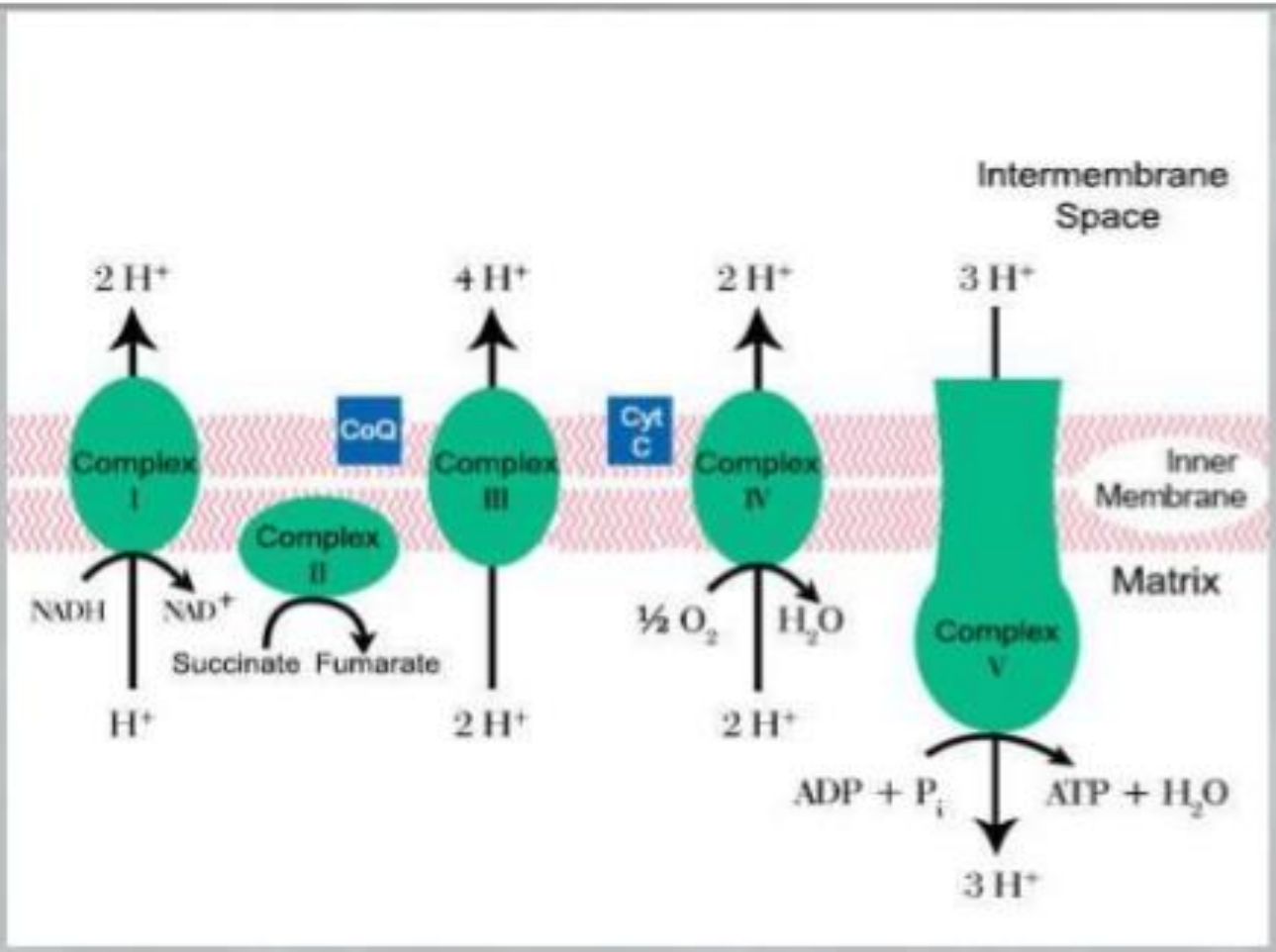
Figure 8-7 Biology: Life on Earth, 8/e


Pyruvate
ATP, acetyl CoA and NADH
Acetyl CoA
Oxalo-acetate
Citrate
Isocitrate
ATP and I NADH
ADP
α-Ketoglutarate
ATP, succinyl CoA, and NADH
Succinyl CoA
Succinate
Fumarate
Malate

Electron Transport Chain

- Last phase of respiration which generate ATP from reduced substrates.
- Consists of a sequence of carrier molecules through which electron passes.
- Occurs in **plasma membrane**.
- Electron transport chain is different in different bacteria.

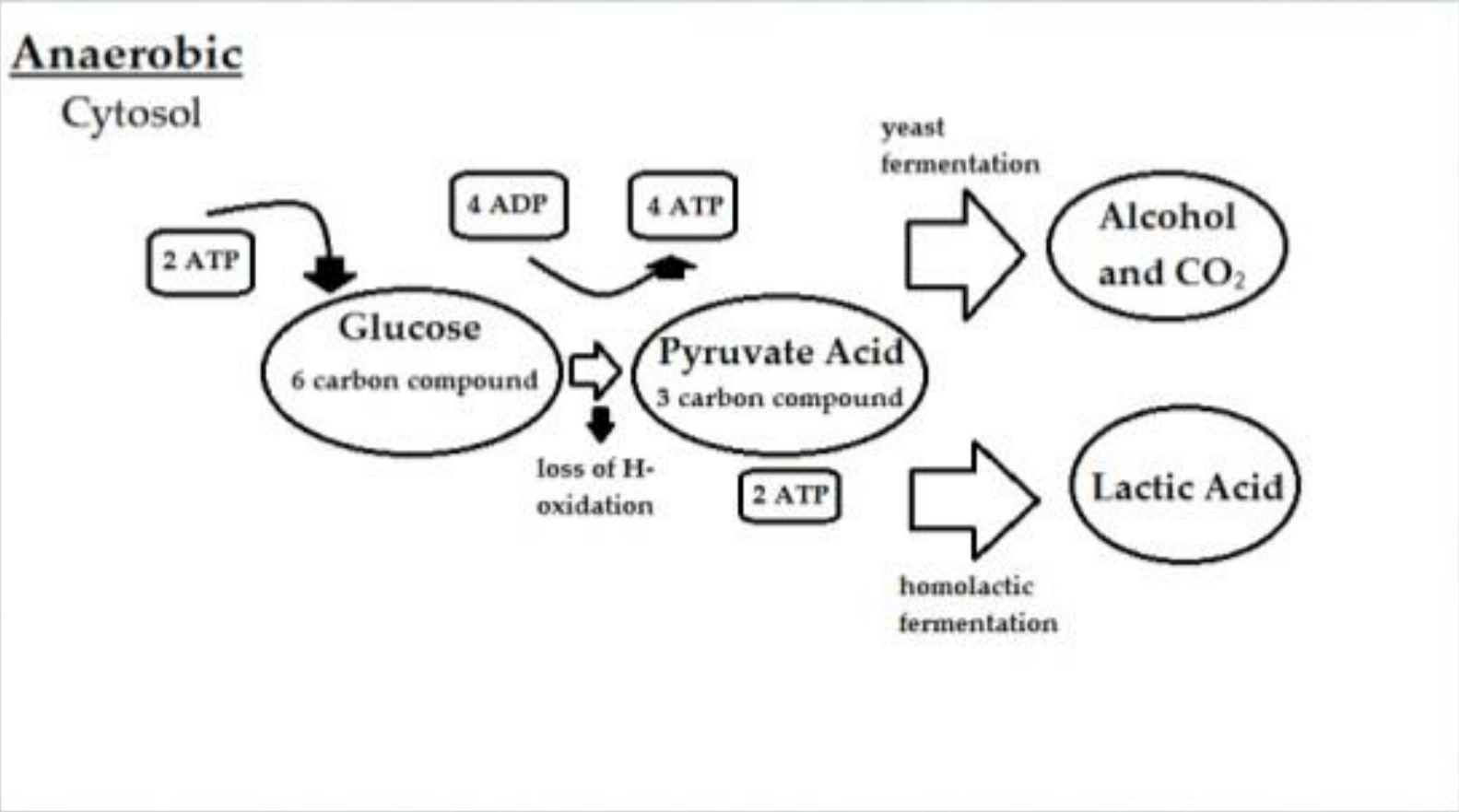
Electron Transport Chain



Fermentation

- Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound.
- In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain.

Anaerobic respiration



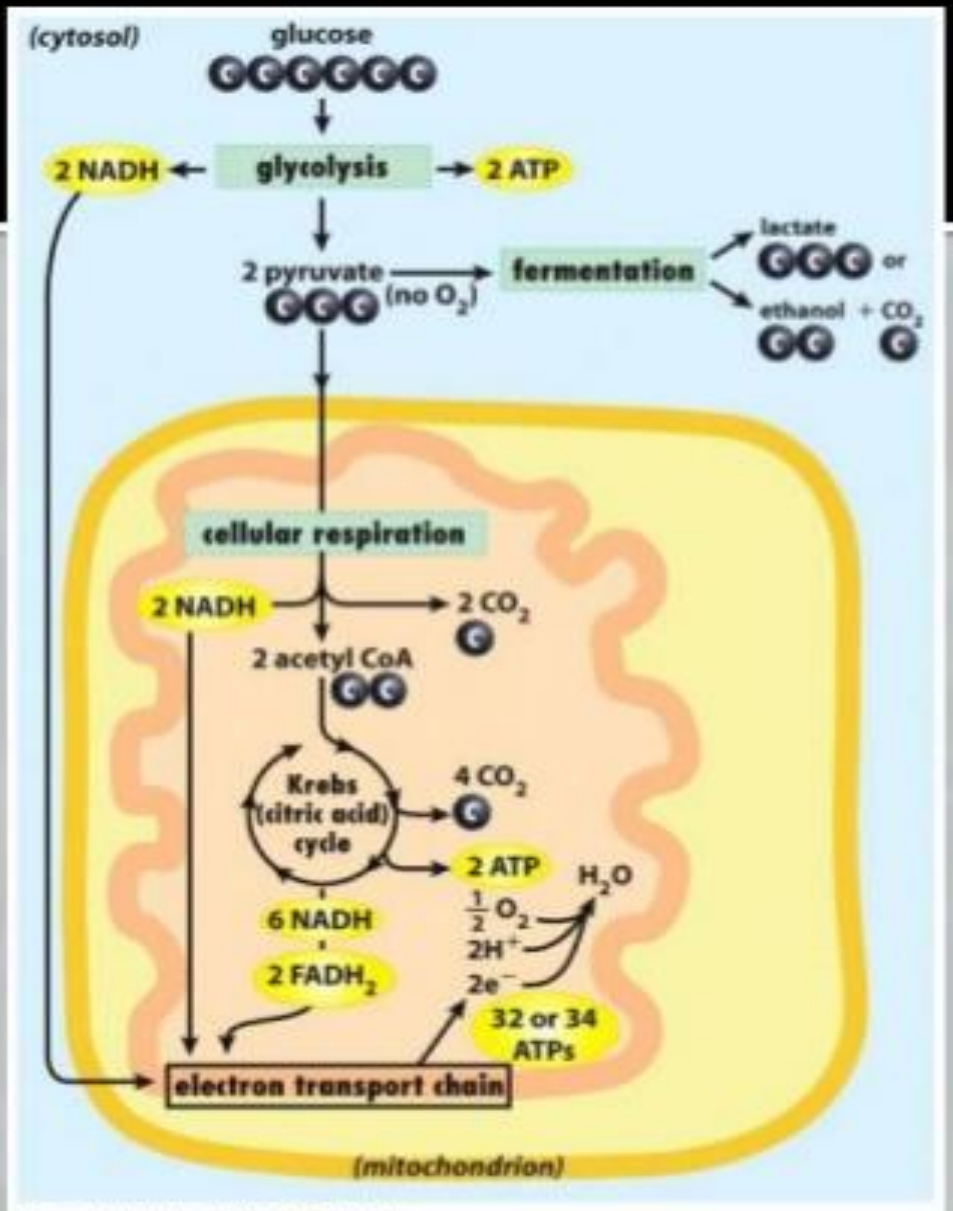


Figure 8-9 Biology: Life on Earth, 8/e

Microbiology of oral cavity

- The colonization of the oral cavity starts close to the time of birth.
- Within hours after birth, the sterile oral cavity will be colonized by low numbers of mainly facultative and aerobic bacteria.
- It is estimated that more than 700 different species are capable of colonizing the adult mouth and that any individual typically harbours 150 or more different species. (Moore WE, 1994)
- Most oral bacteria are harmless commensals under normal circumstances.

Complexity of oral flora

Oral Microflora ref.1-19

Gram(+)		Gram(-)	
Facultative anaerobes	Anaerobes	Facultative anaerobes	Anaerobes
Streptococcus	***Peptostreptococcus***	***Neisseria***	***Veillonella***
S. mutans	***Peptococcus***	***Branhamella***	*V. alcalescens*
S. sanguis	***Streptococcus***		*V.atypica*
S. salivarius			*V. paruvula*
S. milleri			
S. mitis			
Micrococcus			
Actinomyces	***Actimyces***	***Actinobacillus***	***Bacteroides***
A. naeslundii	*A. israelii*	*A. actimycetem-*	*B. gingivitis*
A. viscosus	*A. odontolyticus*	*comitans*	*B. intermedius*
Bacterionema	***Arachinia***	***Capnocytophaga***	*B. forthythus*
Rothia	***Eubactrium***	*C. gingivalis*	*B. melanino.*

- **Biofilm** is an association of micro-organisms in which microbial cells adhere to each other on a living or non-living surfaces within a self-produced matrix of extracellular polymeric substance.

- Biofilm formation is a multi-step process starting with attachment to a surface then formation of micro-colony that leads to the formation of three dimensional structure and finally ending with maturation followed by detachment.

- The dental plaque biofilm also has similar structure.

- The term 'Biofilm' was coined by Bill Costerton in 1978.

- The term Biofilm (Wilderer and Charaklis 1989) describes the relatively indefinable microbial community associated with a tooth surface or any other hard non-shedding material, randomly distributed in a shaped matrix or glycocalyx.

- Biofilm is "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription." **Donlan and Costerton 2002**