Chapter 1. Basics of Microbiology

All the figures and tables in this material are from the reference below unless specified otherwise.
Reference: Bruce E. Rittmann and Perry L. McCarty, "Environmental Biotechnology: Principles and Applications", McGraw-Hill, 2001.

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Intro: Environmental Biotechnology

√ Environmental Biotechnology

i) applies the principles of microbiology to solve environmental problems.
(e.g., water & wastewater treatment, energy production from organic waste)

ii) links the principles of microbiology with engineering fundamentals involving reaction kinetics & engineering as well as conservation of energy & mass.

iii) is always concerned with mixed culture, open, nonsterile systems.
(the major difference between environmental biotechnology and other disciplines that feature biotechnology)

iv) can be successful depending on how individual microorganisms with desired characteristics can survive in competition with other organisms.

- How desired functions can be maintained in complex ecosystems.

- How the survival and proliferation of undesired microorganisms can be prevented. (e.g., Filamentous bacteria in Activated Sludge Process)

1.1 The Cell

√ **Cell:** **the fundamental building block of life,**
an entity separate from other cells and its environment

√ Essential components of cells:

- **Cell wall: a structure that confers rigidity to the cell and protects the membrane**
- **Cell membrane: a barrier between the cell and the environment**
- **Cytoplasm (세포질): water and macromolecules that the cell needs to function**
- **Chromosome (염색체):**

  -A genetic element carrying genes essential to cellular function.

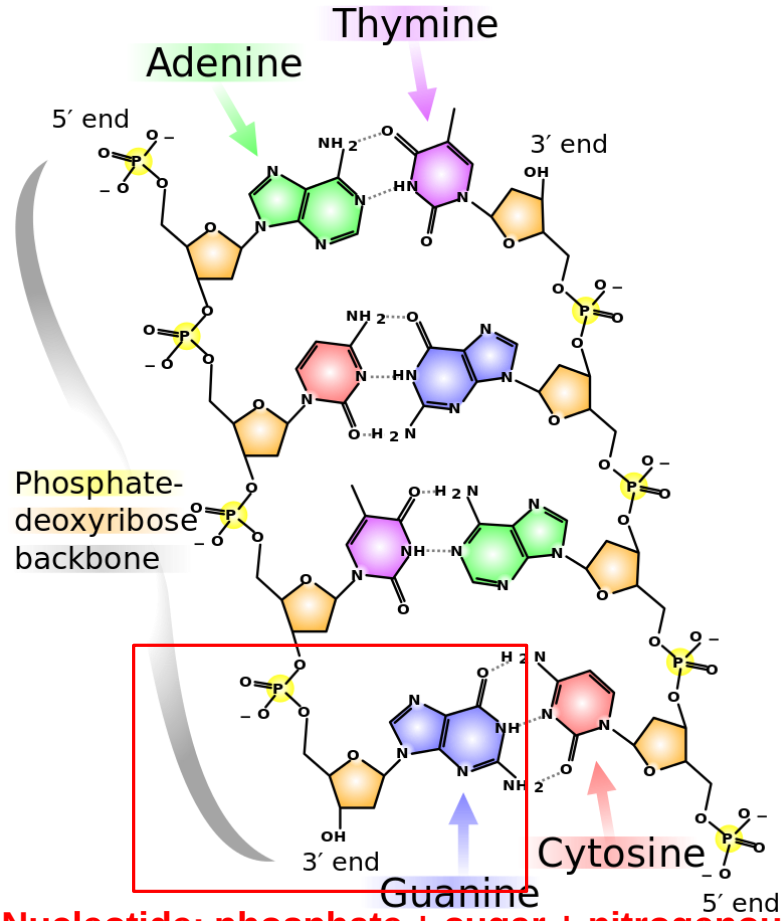
  -Prokaryotes typically have a single chromosome consisting of a circular DNA molecule.

  -Eukaryotes typically have several chromosomes, each containing a linear DNA molecule.

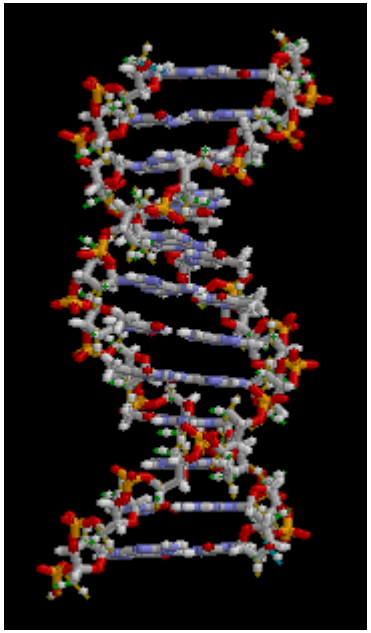
- **Ribosomes (리보솜) :convert the genetic code into proteins (mainly enzymes) that carry out the cell's reactions**
- **Enzymes :catalysts that carry out the desired biochemical reactions.**

1.1 The Cell

- **DNA: Deoxyribonucleic acid**
  DNA stores and replicates the genetic information.



Nucleotide: phosphate + sugar + nitrogenous base



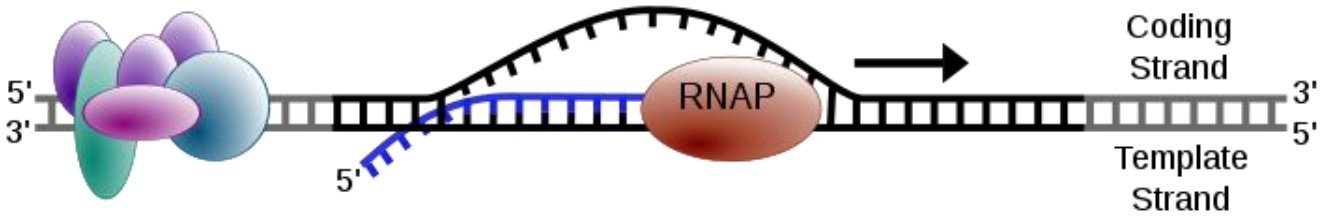
Double stranded helix structure

Source: Wikipedia

1.1 The Cell

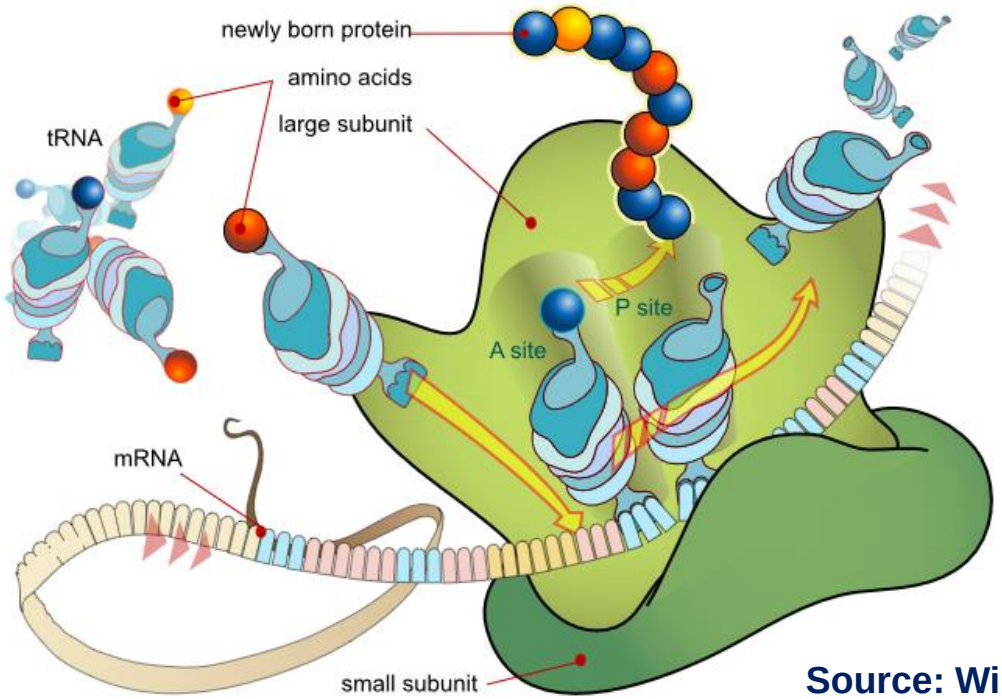
- **Transcription**

  : the process in which a particular segment of DNA is copied (transcribed) into RNA (ribonucleic acid) by the enzyme RNA polymerase (RNAP)



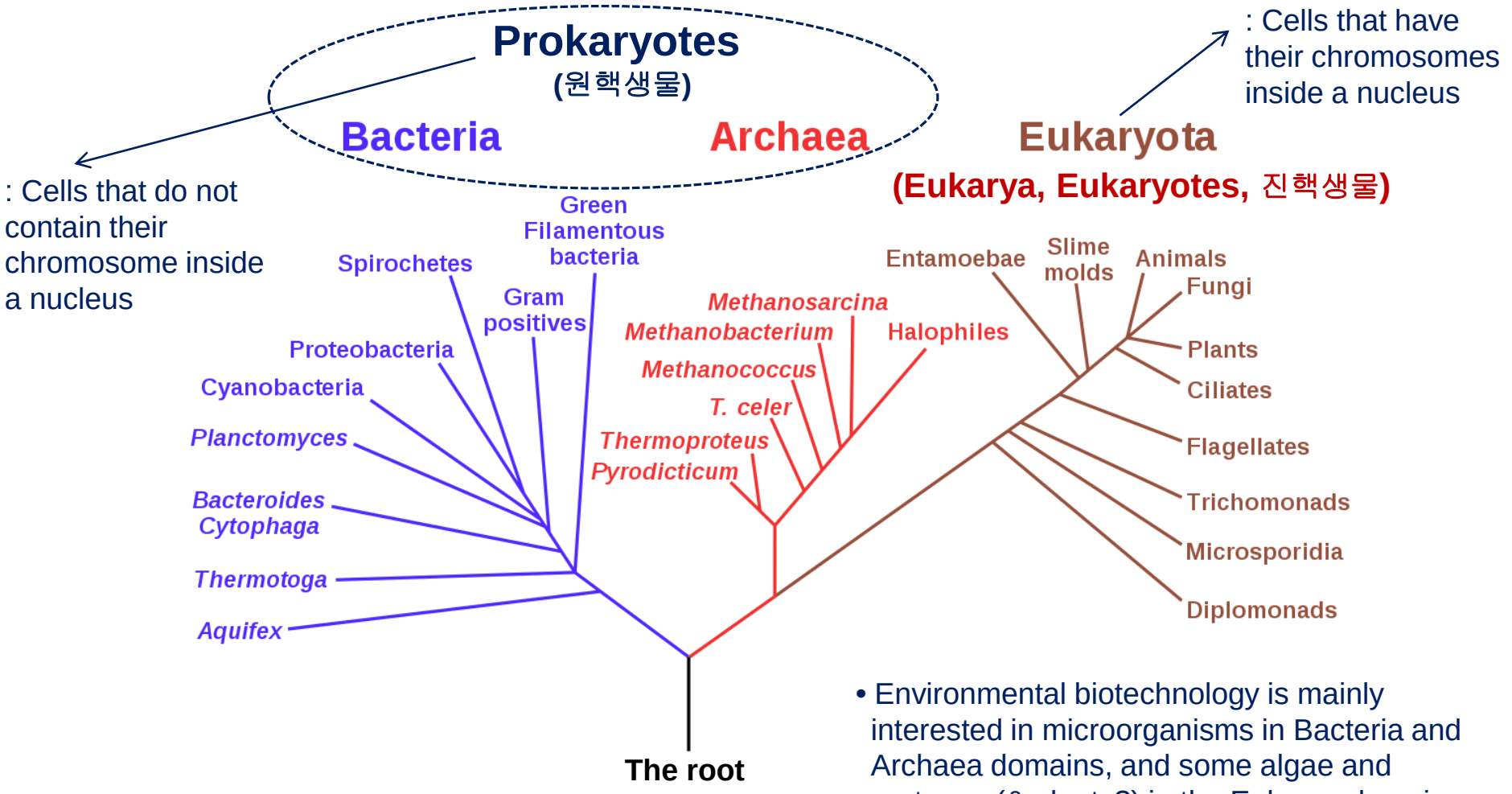
- **Translation**

  : the process in which ribosomes synthesize proteins



Source: Wikipedia

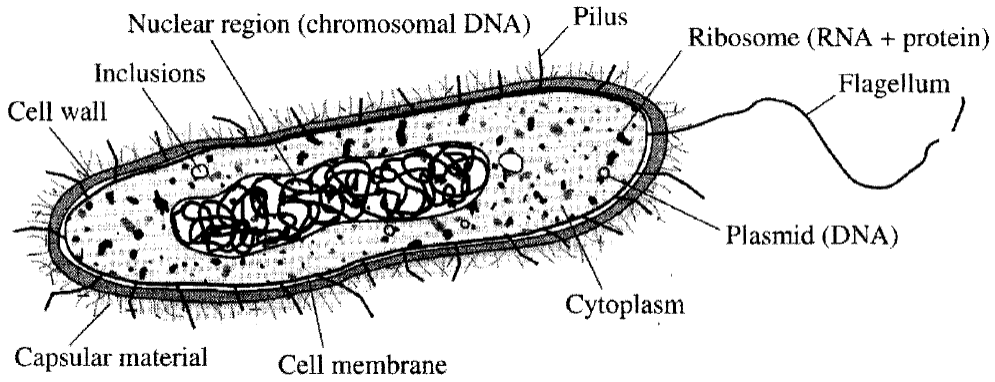
1.1 The Cell



- Environmental biotechnology is mainly interested in microorganisms in Bacteria and Archaea domains, and some algae and protozoa (& plants?) in the Eukarya domain.

▲ **Phylogenetic tree of life as determined from comparative rRNA sequencing**

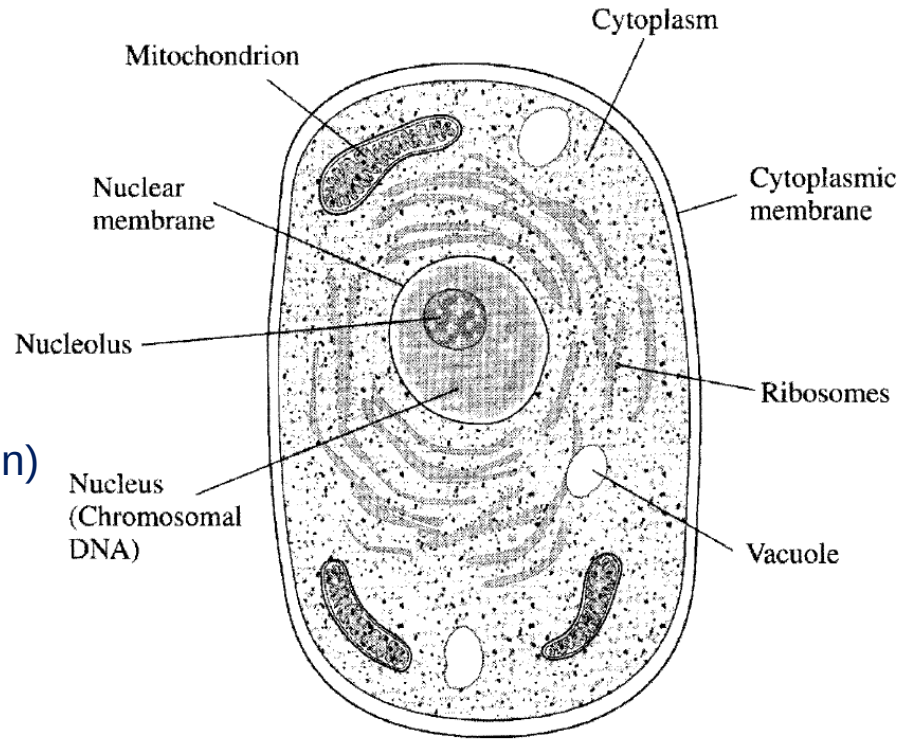
1.1 The Cell



▲ **Prokaryotic cells**

- Simple and small (0.1–5 μm), ancient cells
- Lack of organelles (such as nucleus, mitochodrion)

They both have DNA, ribosomes, cytoplasm, cell membranes (cytoplasmic or plasma membranes).



▲ **Eukaryotic cells**

- Complex and large (10–100 μm), evolved cells
- Many membrane-bound organelles

1.2 Taxonomy and Phylogeny

√ Taxonomy

- Taxis (arrangement) + Nomos (law)

- Taxonomy relies on observable properties (phenotype)
  i) morphology,
  ii) transformation (ability to use or convert a given chemical into another one)
  iii) Staining (the manner in which it interacts with dyes)

e.g., taxonomic ranks of ***Pseudomonas fluorescens***

Formal rank	Example
Domain	*Bacteria*
Division (Phylum)	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Pseudomonadales*
Family	*Pseudomonadaceae*
Genus	*Pseudomonas*
Species	***Pseudomonas fluorescens***

1.2 Taxonomy and Phylogeny

√ Phylogeny

- Phylogeny is the method of classification based on genetic characteristics (encoded in DNA and RNA).
- The sequences of base pairs in an organism's 16S ribosomal RNA (rRNA) are important (used to distinguish two domains, the Bacteria and the Archaea).

  * *The number 16S refers to Svedberg units, which are units of sedimentation coefficients of ribosome subunits or intact ribosomes when subjected to centrifugal force in an ultracentrifuge.*

- Phylogeny relates organisms based on their evolution history, while taxonomyrelates organisms based on observable characteristics of the cells.

1.2 Taxonomy and Phylogeny

√ Prokaryotes

- Cells that do not contain their chromosome inside a nucleus
- Single cellular organisms
- Bacteria (the cell wall contains peptidoglycan (murein))

  Archaea (it does not)

√ Eukaryotes

- Cells that have their chromosomes inside a nucleus
- Single cellular (algae & protozoa) or multi-cellular organisms

1.2 Taxonomy and Phylogeny

Table 1.1 Differing features among Bacteria, Archaea, and Eukarya

peptidoglycan

Characteristic	Bacteria	Archaea	Eukarya
Membrane-enclosed nucleus	Absent	Absent	Present
Cell wall	Muramic acid present	Muramic acid absent	Muramic acid absent
Chlorophyll-based photosynthesis	Yes	No	Yes
Methanogenesis	No	Yes	No
Reduction of S to H_2S	Yes	Yes	No
Nitrification	Yes	No	No
Denitrification	Yes	Yes	No
Nitrogen fixation	Yes	Yes	No
Synthesis of poly-β-hydroxyalkanoate carbon storage granules	Yes	Yes	No
Sensitivity to chloramphenicol, streptomycin, and kanamycin	Yes	No	No
Ribosome sensitivity to diphtheria toxin	No	Yes	Yes

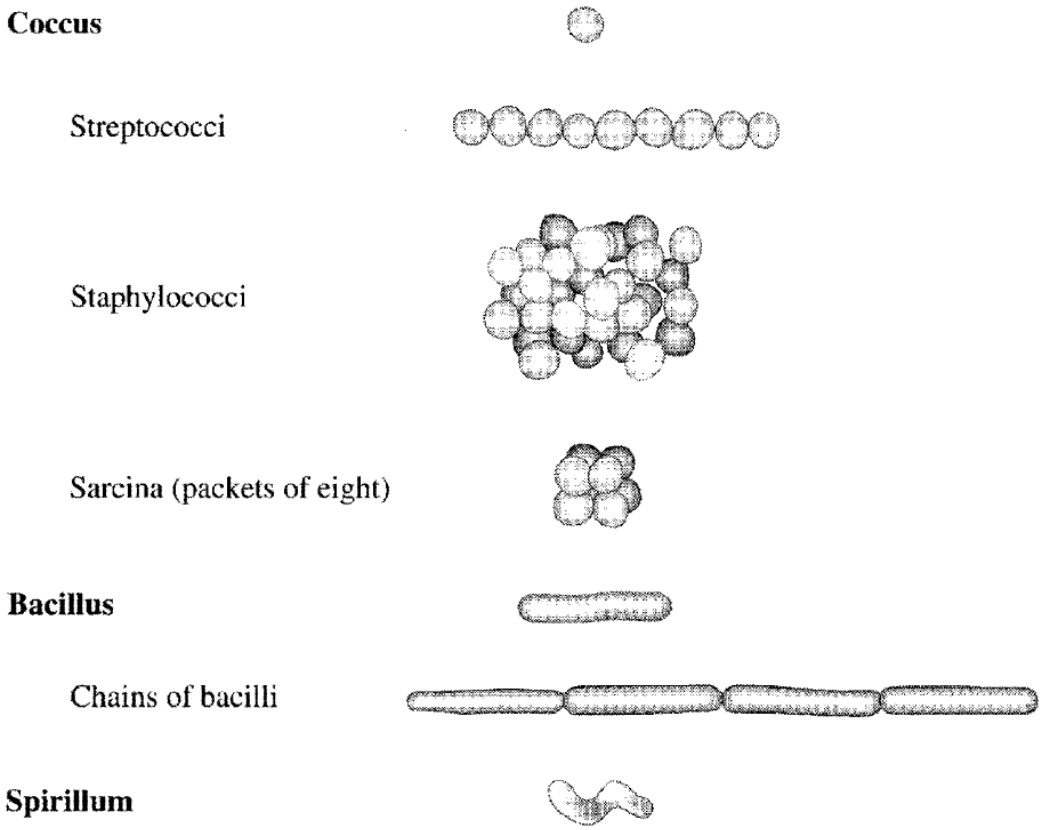
SOURCE: Madigan, Martinko, and Parker, 1997.

1.3 Prokaryotes – Bacteria

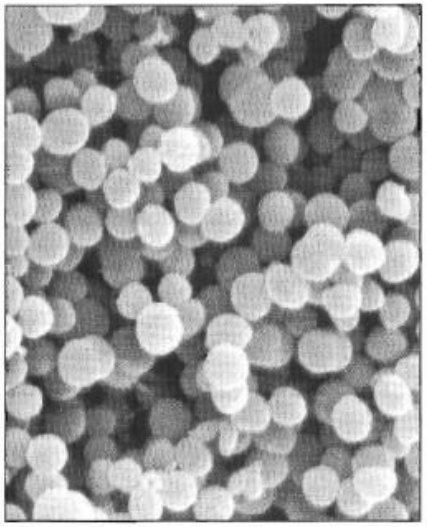
√ Morphology

: Shape, size, structure, spatial relationship to one another

Shape: Coccus (spherical), Bacillus (rods), Spirillium (helical shape)



1.3 Prokaryotes – Bacteria



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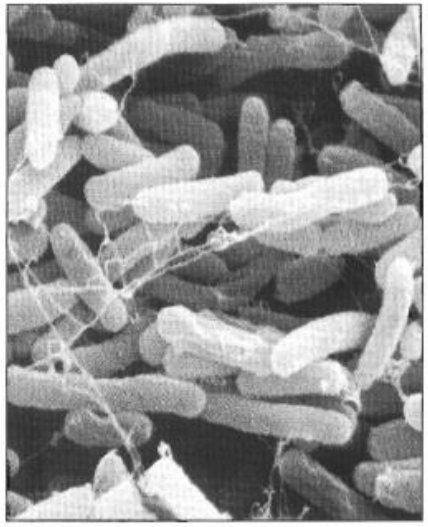

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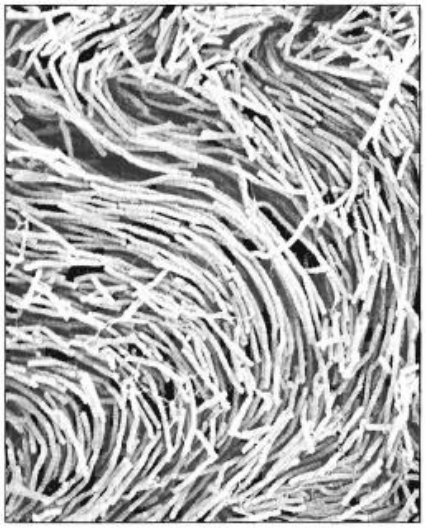

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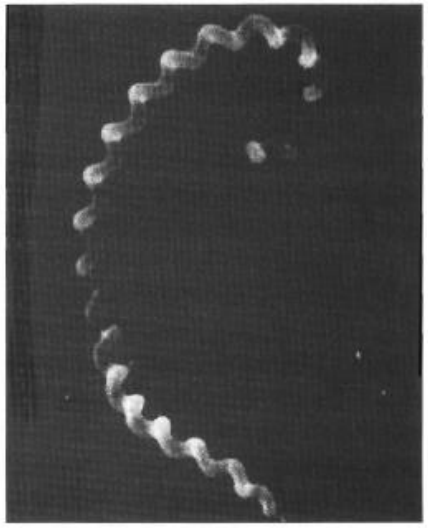


d.

Figure 1.3

Scanning electron microphotographs of typical bacteria. *a. Staphylococcus epidermidis. b. Escherichia coli. c Bacillus* chains. *d. Leptospyra interrogans.* SOURCE: With permission of the Microbe Zoo and Bergey's Manual trust, Michigan State University. Images *a, b, c* were created by Shirley Owens, and image *d* is from *Bergey's Manual of Systematic Bacteriology*, Vol. 1, Williams and Wilkins, Baltimore (1984).

1.3 Prokaryotes – Bacteria

√ EPS (Extracellular Polymeric Substances)

- Organic polymers of microbial origin which in biofilm systems are frequently responsible for binding cells and other particulate materials together (cohesion) and to the substratum (adhesion)

  **They are of much significance in environmental biotechnology*

- They are excreted from the cell and do not diffuse away from the cell because of viscous nature

- They increase the viscosity of the surrounding fluid and can also help to hold bacteria together in aggregates or bacterial “flocs”.

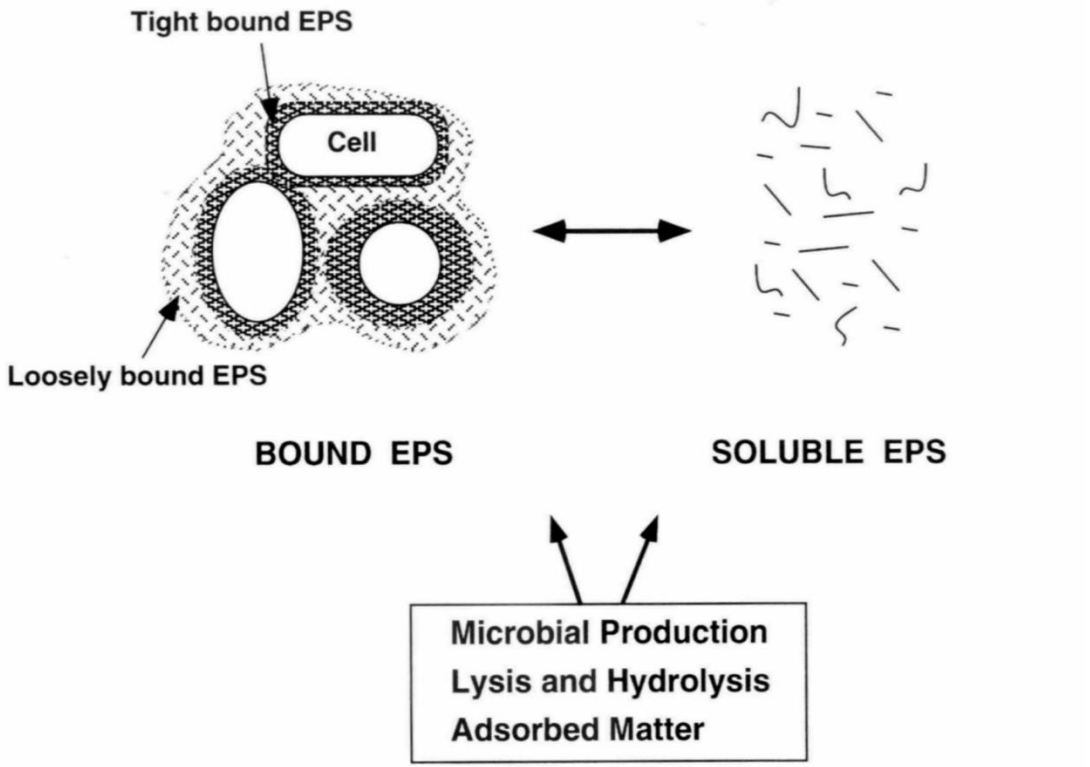
- They form capsule or slime layer (= biofilm).

- Different classes of organic molecules such as polysaccharide, proteins, nucleic acids, (phospho)lipids, and other polymeric compounds, which have been bound to occur in the intercellular spaces of microbial aggregates.

- Important to stress that EPS is composed by a number of different organic macromolecules of different origin (humic substances attached). it is basically impossible to perform any extraction that extracts only the exopolymers produced by the microbes present in the aggregate.

1.3 Prokaryotes – Bacteria

√ EPS (Extracellular Polymeric Substances)



-Physiological states of activated sludge

-Architecture of bio-cake

-Membrane permeability

▲ **Definition of cell biomass and extracellular polymeric substances(EPS)**

*The EPS may arise from microbial production, lysis and hydrolysis, or from attachment ,e.g., substances from water phase to bioaggregates

1.3 Prokaryotes – Bacteria

√ **Chemical composition**

Table 1.2 Chemical and macromolecular characteristics of prokaryotic cells

Chemical Composition

Constituent	Percentage
Water	75
Dry Matter	25
Organic	90
C	45–55
O	22–28
H	5–7
N	8–13
Inorganic	10
P_2O_5	50
K_2O	6.5
Na_2O	10
MgO	8.5
CaO	10
SO_3	15

1.3 Prokaryotes – Bacteria

Macromolecular Composition		*E. coli* and *S. typhimurium*[a]	
	Percentage[b]	**Percentage**	**Molecules per cell**
Total	100	100	24,610,000
Proteins	50–60	55	2,350,000
Carbohydrates	10–15	7	
Lipids	6–8	9.1	22,000,000
Nucleic Acids			
DNA	3	3.1	2.1
RNA	15–20	20.5	255,500

[a]Data from Madigan, Martinko, and Parker (1997) and G.C. Neidhardt et al. (1996). *E. coli* dry weight for actively growing cells is about 2.8×10^{-13} g.
[b]Dry weight.

1.3 Prokaryotes – Bacteria

√ Reproduction and Growth

- Bacteria normally reproduce through binary fission, in which a cell divides into two. This asexual reproduction occurs spontaneously after a growing cell reaches a certain size.

- Generation time: the interval of time required for the formation of two cells from one

  - It may be as short as 30 min, as with *E.coli*.

  - The weight would increase from 10-13g to that of a human child (18kg) in a single day

**Environmental and nutritional limitations of the culture flask generally limit the growth long before such a mass increase occurs,*
but the potential for such rapid growth is inherent in the bacterial cell.

1.3 Prokaryotes – Bacteria

√ Classes of bacteria based on energy and carbon-source

- **Phototrophs:** use energy from light
- **Chemotrophs:** obtain energy from chemical reactions
  - **Chemoorganotrophs:** use organic chemicals for energy
  - **Chemolithotrophs:** use inorganic chemicals for energy

- **Autotrophs:** use inorganic carbon (e.g.,CO_2) for cell synthesis
- **Heterotrophs:** use organic compounds for cell synthesis.

1.3 Prokaryotes – Bacteria

√ Environmental condition for growth

- **Temperature**

  Psychrophile (-5~20℃), Mesophile (8~45℃),

  Thermophile (40~70℃), Hyperthermophile (65~110℃)

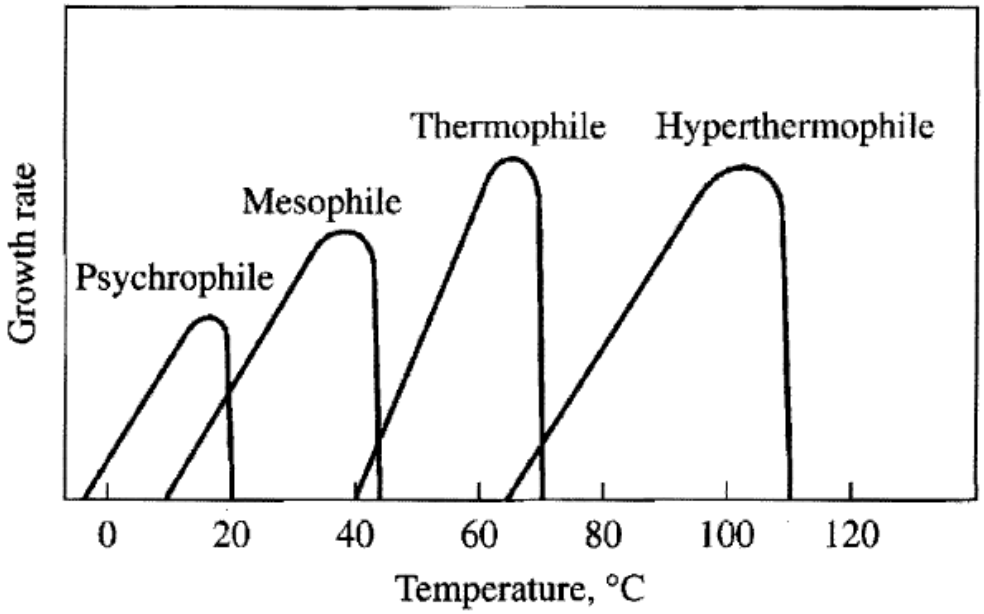


Figure 1.5 Effect of temperature on growth rate of different temperature classes of bacteria.

- **pH**

- pH range for growth: 6~8 for most bacteria

- **Oxygen**

- Aerobic, Anaerobic

- **Salt**

- Halophiles (3.5% NaCl), Extreme halophile (10~30% NaCl)

1.3 Prokaryotes – Bacteria

√ Proteobacteria = Purple bacteria

- The most studied and important group of bacteria for environmental biotechnology
- Traditional Gram-negative bacteria
- Largest number of species
- Alpha, beta, gamma, delta, epsilon subgroup (by 16S rRNA)
- Diverse physiology: phototrophic, chemolithotropic, chemoorganotrophic
- They have ability to transform a great variety of inorganic and organic pollutants into harmless minerals.
- They are the causative agents of disease (Pathogens)

1.3 Prokaryotes – Bacteria

Table 1.4 Major groupings among the purple bacteria and the common genera for each group

Alpha	*Rhodospirillum,* Rhodopseudomonas,* Rhodobacter,* Rhodomicrobium,* Rhodovulum,* Rhodopila,* Rhizobium, Nitrobacter, Agrobacterium, Aquaspirillum, Hyphomicrobium, Acetobacter, Gluconobacter, Beijerinckia, Paracoccus, Pseudomonas* (some species)
Beta	*Rhodocyclus,* Rhodoferax,* Rubrivivax,* Spirillum, Nitrosomonas, Sphaerotilus, Thiobacillus, Alcaligenes, Pseudomonas, Bordetella, Neisseria, Zymomonas*
Gamma	*Chromatium,* Thiospirillum,** other purple sulfur bacteria,* *Beggiatoa, Leucothrix, Escherichia* and other enteric bacteria, *Legionella, Azotobacter,* fluorescent *Pseudomonas* species, *Vibrio*
Delta	*Myxococcus, Bdellovibrio, Desulfovibrio* and other sulfate-reducing bacteria, *Desulfuromonas*
Epsilon	*Thiovulum, Wolinella, Campylobacter, Helicobacter*

*Phototrophic representatives.
SOURCE: Madigan, Martinko, and Parker, 1997.

1.3 Prokaryotes – Archaea

- Bacteria : The cell wall contains peptidoglycan
  Archaea : The cell wall does not contain peptidoglycan

- Microorganisms that convert acetate and hydrogen to methane (methanogens).

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

- Archaea are characterized by a large number of extremophiles, organisms living under extreme environmental conditions (thermophiles, halophiles, etc.)

Table 1.5 Major groups and subgroups for the Archaea

Group	Subgroups
Crenarchaeota	*Desulfurococcus, Pyrodictium, Sulfolobus, Thermococcus, Thermoproteus*
Korarchaeota	
Euryarchaeota	*Archaeroglobus, Halobacterium, Halococcus,* Halophilic methanogen, *Methanobacterium, Methanococcus, Methanosarcina, Methanospirillum, Methanothermus, Methanopyrus, Thermoplama*

1.4 Eukarya (Eukaryotes) – Fungi

√ Fungi

- All are organolithotrophic, and none are phototrophic.

- They are known to decompose a great variety of organic materials that tend to resist bacterial decay. They have the key oxidative enzyme (peroxidase), which helps to break lignins.
  e.g., leaves, dead plants, dead trees, and other lignocellulosic organic debris.

- They are specially important for degradation of toxic compounds.

- But, slow decomposition rate → making less attractive for engineered systems

1.4 Eukarya (Eukaryotes) – Algae

√ Algae

- Oxygenic phototrophs
  the main source of oxygen in natural water bodies

- Problems
  - Production of taste and odor compounds, toxins in water supplies
  - Filter clogging at water treatment plants
  - Decreased clarity of lakes (produces turbidity)

- Most species are phototrophs, autotrophs.

- As algae grows, pH tends to increase

  e.g., $HCO_3^- + H_2O \rightarrow CH_2O + O_2 + OH^-$

- A pH greater than 8.5 to 9 is detrimental to algae growth.
  (a few species can continue to extract inorganic carbon from water until pH reaches 10–11.)

- Algae generally prefer a near neutral to alkaline pH.

- Algae (C:50%, N: 10%, P:2%)
  Under nitrogen or phosphorous limiting conditions, the rate of Algae growth is reduced.

1.4 Eukarya (Eukaryotes) – Protozoa

√ Protozoa

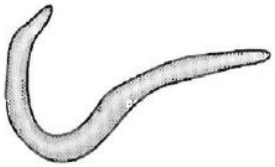
- Single-celled, heterotropic eukaryotes that can pursue and ingest their food.
- Generally feed on bacteria and other small particulate matter.
- Can be differentiated according to their method of locomotion.
- In biological treatment systems, protozoa act to polish effluent streams by helping to cleanse them of fine particulate materials that would otherwise leave in the effluent.
  - These organisms ingest colloidal and soluble organic material and typically help to clarify secondary effluent. In their absence colloid concentrations are higher.

Group	Common name	Locomotion
Sarcodina	Ameba	Pseudopodia
Masigophora	Flagella	One or more flagella
Ciliophora	Ciliates	Cilia
Sporozoa		nonmotile

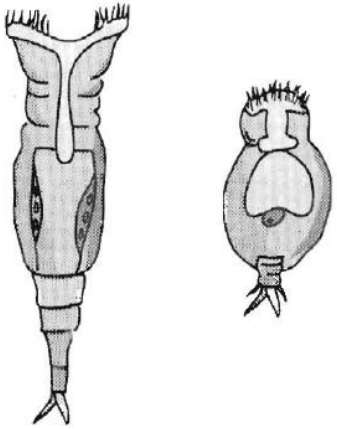
1.4 Eukarya (Eukaryotes) – Other Multicellular Organisms

- Mutulcellualr, strictly aerobic and ingest small particulate organic matters (bacteria, algae, other living or dead organic particles of similar size).

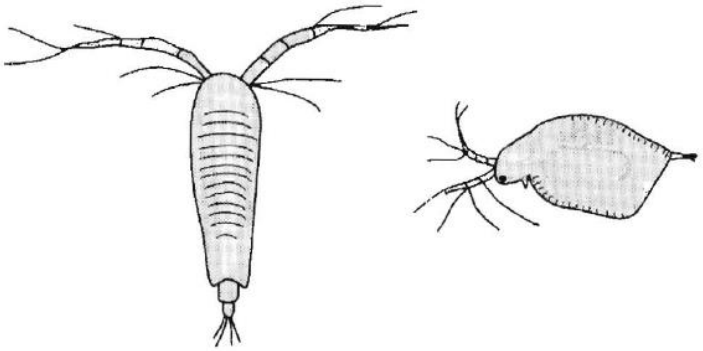
Nematodes



Rotifers



Crustacea



1.5 Viruses

- Viruses are generally not considered to be "living" entities, as they are unable, on their own, to replace their parts or to carry out metabolism.

- Viruses range in size from about 15-300 nm.

- Bacteriophages

  - Viruses that infect prokaryotic cells

  - They are prevalent in biological wastewater treatment systems.

  - When bacteria are infected with phages, the bacteria cell bursts open, releasing them for infectious of other cells. So they have been suspected of causing process upsets by killing needed bacteria.

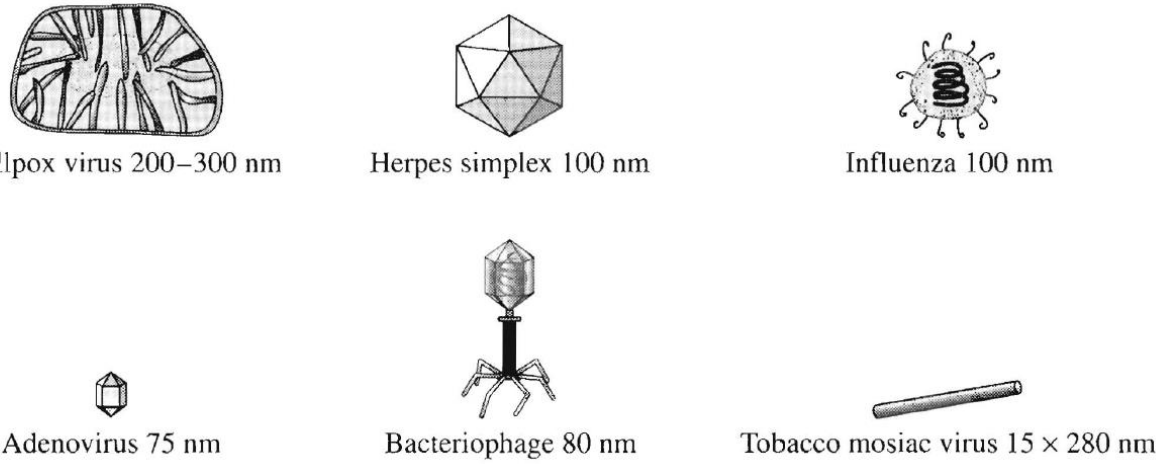


Figure 1.10 Typical structures of viruses.

1.6 Infectious Disease

Table 1.9 Causative agents of waterborne and/or human-waste-related disease

Microorganism Class	Group	Organism Name	Disease and Symptoms
Virus	Viscerotropic	Coxsackie virus, Norwalk virus, Rotavirus, Echovirus	Gastroenteritis
	Viscerotropic	Hepatitis A virus	Infectious hepatitis, liver inflammation
	Neurotrophic	Polio virus	Poliomyelitis
Bacteria (Purple Group)	Epsilon	*Campylobacter jejuni*	Gastroenteritis, diarrhea, fever, abdominal pain
	Epsilon	*Helicobacter pylori*	Peptic ulcers
	Gamma	*Escherichia coli* O157:H7	Diarrhea, hemorrhagic colitis
	Gamma	*Legionella pneumophilia*	Legionellosis, fever, headache, respiratory illness
	Gamma	*Salmonella typhi*	Typhoid fever, blood in stools
	Gamma	*Shigella dysenteriae*	Dysentery, abdominal cramps, bloody stools
	Gamma	*Vibrio cholerae*	Cholera, severe diarrhea, rapid dehydration
Algae	Dinoflagellate	*Gambierdiscus toxicus*	Ciguatera fish poisoning
	Dinoflagellate	*Gonyaulax catanella*	Shellfish poisoning
	Dinoflagellate	*Pfiesteria piscicida*	Fish poisoning, memory loss, dermatitis
Protozoa	Mastigophora	*Giardia lamblia*	Giardiasis, diarrhea, bloating
	Sarcodina	*Entamoeba histolytica*	Amebiasis, sharp abdominal pain, bloody stools
	Sporozoa	*Cryptosporidium parvum*	Cryptosporidiosis, diarrhea
Multicellular parasites		*Schistosoma mansoni*	Schistosomiasis, fever, diarrhea, dermatitis

1.6 Infectious Disease

√ Protozoan-related diseases

• Giardia lamblia (Giardiasis –a foul smelling diarrhea)

- Giardia cysts are resistant to disinfection.

- Warning to wilderness campers to boil or filter water taken from seemingly pristine environments.

- 1965-81, 53 waterborne outbreaks of Giardiasis in USA (20,000 people were affected)

• Cryptosporidium parvum

- C. Parvum oocysts is highly resistant to chlorination.

- In 1993, about 370,000 people in Milwaukee, Wisconsin, developed a diarrheal illness (100 died)

1.7 Biochemistry

√ **Biochemistry:**

the study of chemical processes (reactions) within and relating to living organisms

- Microorganisms grow at the expense of potential energy stored in inorganic or organic molecules or present in radiant energy (light)

- In order to obtain the energy, organisms transform the chemicals through oxidation and reduction reactions.

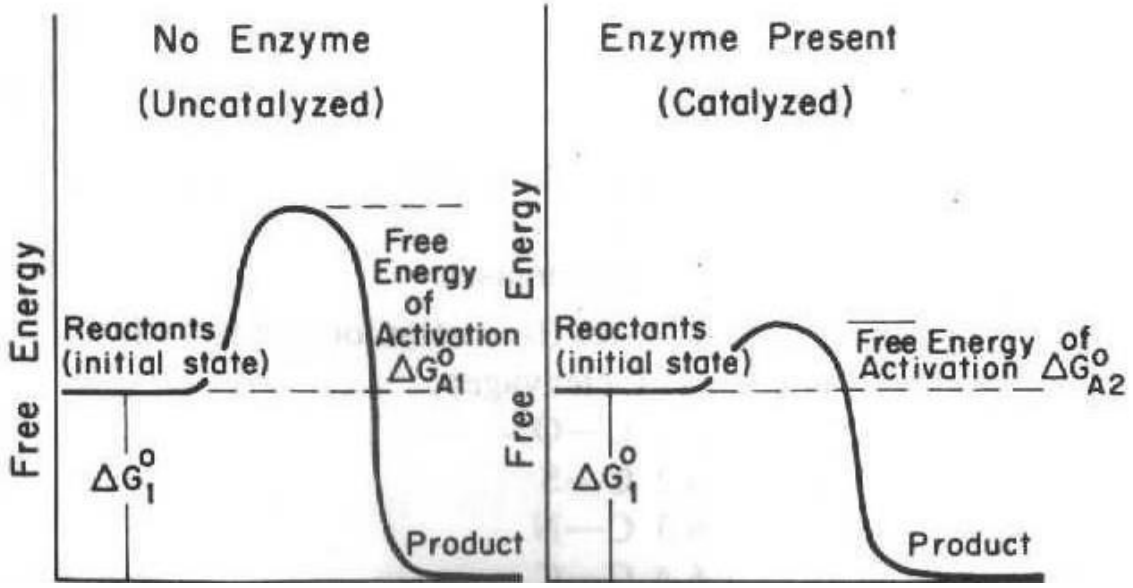
  - Enzymes catalyze all the key reactions

- Only through an understanding of the microorganisms' energy-yielding and energy-consuming reactions (biochemistry), we can create the conditions that lead to environmental protection and improvement.

1.8 Enzymes

√ **Enzymes:** **Organic catalysts produced by microorganisms**

- Speed up the rate of the thousands of energy-yielding and cell-building reactions



- The largest and most specialized group of protein molecules within the cell.
- **M.W:** generally 10,000 ~ a million
- **Structure:** primary, secondary, and tertiary structure
- **Enzyme denaturation or inactivation:** by heat or chemicals

1.8 Enzymes

- **Nomenclature : commonly named by adding the suffix *–ase***

  e.g., *dehydrogenase, hydroxylase, proteinase, oxido-reductase,* etc.

- **Major source of energy :** Redox (reduction & oxidation) reactions

- **Redox reactions involve the transfer of electrons**
  - Primary electron donor
  - Electron carrier (shuttle)
  - Terminal electron acceptor

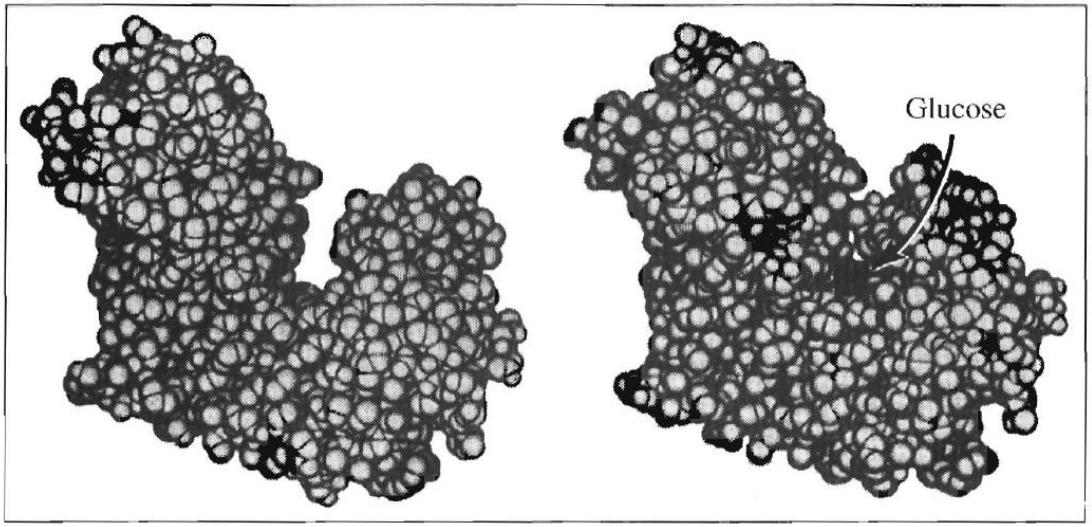
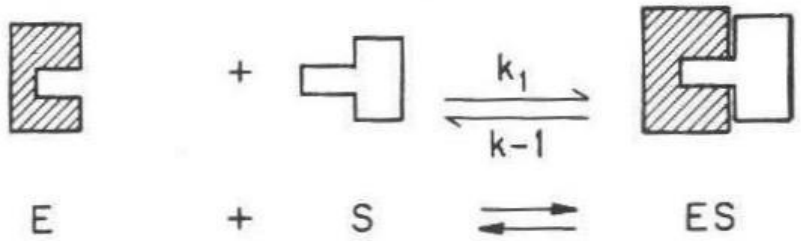


Figure 1.11 Computer-generated structure of the enzyme hexokinase that converts glucose and ATP into glucose 6-phosphate during the first step of glycolysis. Shown is the location where the glucose molecule fits into the enzyme structure and the resulting change in the enzyme configuration. Courtesy of Dr. Thomas Steitz.

1.8 Enzymes – Enzyme Reactivity

√ **Enzyme reaction:** lock-and-key fashion



- The rate (or kinetics) of an enzyme-catalyzed reaction is governed by the same principles that govern other chemical reactions.

- The complicated biological mechanisms discussed are rarely known in sufficient detail to allow formulation of an analytical kinetic expression.

- Certainly in wastewater treatment where the biomass is bacteriological zoo and the substrates are a mixture of household and industrial wastes, any kinetic expression for the biological reaction rates must be based upon a number of simplifying assumptions.

- Microorganisms are “bags full of enzymes” so that it is not surprising that the growth rate of microorganisms (Monod equation) is related to the reactions of the catalysts (enzymes) that mediate many reactions (Michaelis and Menten equation).

1.8 Enzymes – Enzyme Reactivity

√ Michaelis and Menten model

- **Assumption:**

- The substrate S is reversibly combined with enzyme E to form a complex ES

- The reverse reaction between free product (P) and enzyme (E) is negligible during the initial course of the reaction.

- The transformation of the ES complex into the free product (P) and Enzyme (E) is rate-determining

1.8 Enzymes – Enzyme Reactivity

- **General theory of enzyme reaction and kinetics: Michaelis-Menten equation**

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad [1.3]$$

$$ES \xrightarrow{k_2} E + P \qquad [1.4]$$

- **The rate of formation of ES from equation 1.3:**

$$\frac{dES}{dT} = k_1(E_0 - ES)S \qquad [1.5]$$

E_0 : total enzyme conc. k : rate constant

$E_0 - ES$ = free enzyme conc.

- **The rate of breakdown of ES:**

$$-\frac{dES}{dt} = k_{-1}ES + k_2ES \qquad [1.6]$$

1.8 Enzymes – Enzyme Reactivity

- **At steady state :** equation [1.5] is equal to [1.6]

$$k_1(E_0 - ES)S = k_{-1}ES + k_2ES \qquad [1.7]$$

$$\frac{S(E_0 - ES)}{ES} = \frac{k_{-1} + k_2}{k_1} = K_M \qquad [1.8]$$

$$ES = \frac{E_0 \cdot S}{K_M + S} \qquad [1.9]$$

√ K_M: Michaelis-Menten coefficient

- **affinity between the substrate and the enzyme**
- **a low value of K_M:** strong affinity
- **a high value of K_M:** poor affinity

1.8 Enzymes – Enzyme Reactivity

$$v = dP/dt = -dS/dt = k_2 ES \qquad [1.10]$$

- *v*: the rate of formation of product *P*

$$v = \frac{k_2 E_0 \cdot S}{K_M + S} \qquad [1.11]$$

- **If the substrate concentration is very high (K_M << S)**

$$v_m = k_2 E_0 \qquad [1.12]$$ v_m: maximum velocity

- **Dividing equation 1.11 by 1.12**

$$v = v_m \frac{S}{K_M + S} \qquad [1.13]$$ ⇒ **Michaelis-Menten equation**

- **quantitative relationship between the substrate concentration and the reaction rate**

1.8 Enzymes – Enzyme Reactivity

- **For an important case where v=1/2v_m,**

$$\frac{1}{2} = \frac{S}{K_m + S} \quad [1.14]$$

$$S = K_m, when\ v = \frac{v_m}{2} \quad [1.15]$$

- **Thus, the coefficient, K_m equals the substrate concentration S at which the velocity of the reaction is one-half of the maximum velocity.**

(The unit of K_m = The unit of S)

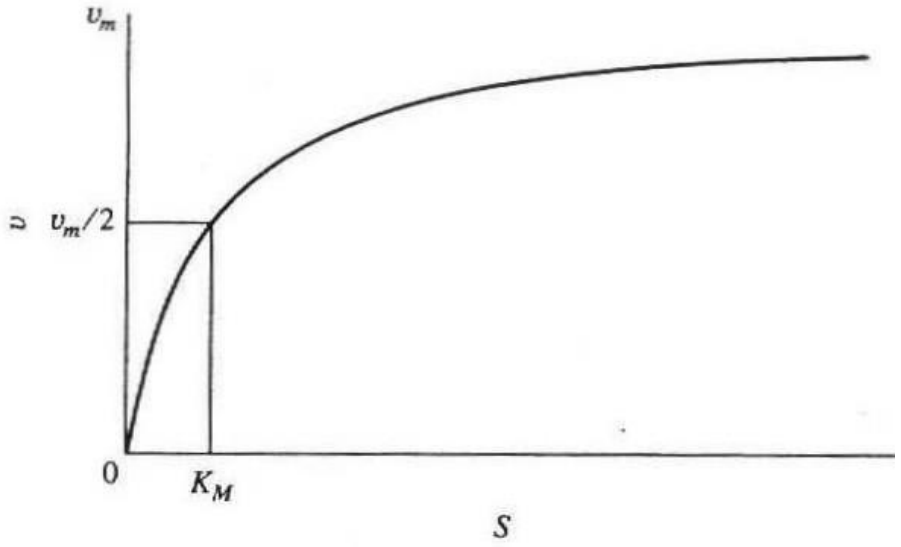


Figure 1.12 Effect of substrate concentration (S) on enzymatic transformation rate (v) based upon Michaelis-Menten kinetics. When S equals K_M, v is one-half of the maximum rate v_m.

1.8 Enzymes – Enzyme Reactivity

- When enzyme transforms more than one substrate, the value for K_m and V_m are different for each substrate.

- For a single substrate–enzyme reaction, K_m is generally 10^{-5} ~ 10^{-2} M. Then, it only requires 10^{-5} ~ 10^{-2} M of S to allow an enzyme to operate half of V_m.

- **If** $\boldsymbol{K_m \gg [S], v = \frac{v_m}{K_m}[S] = K'[S]}$ $\Rightarrow$ **First-order decay**

- **If** $\boldsymbol{K_m \ll [S], v = v_m}$ $\Rightarrow$ **Zero-order decay**

1.8 Enzymes – Enzyme Reactivity

√ Effects of pH and temperature on enzyme reactivity

• pH

- Many enzymes have optimal activity at neutral pH

  The enzyme activity decreases with either increasing or decreasing pH from this optimal point.

- Some others have optima at higher or lower pH values.

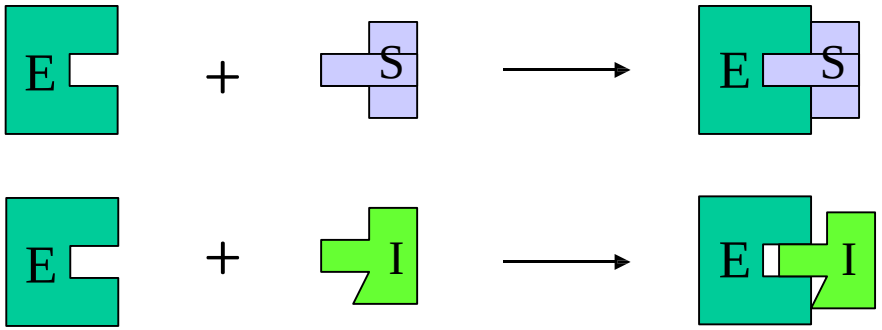
• Temperature

- Rates of reaction roughly double for each 10℃ increase in temperature
- Greater than the optimum temperature: the enzyme begins to denature and the enzyme's activity then deteriorates and ceases.

1.8 Enzymes – Enzyme Reactivity

- Chemical agents reduce the enzyme reactivity
  (So toxic chemicals can adversely affect a biological treatment process.)
- Chemical agents does not destroy the enzyme, and the reactivity can be reversed if the agent is removed (reversible inhibition)
- **Reversible inhibition :** competitive and noncompetitive inhibition

- **Competitive inhibition :** a chemical that is similar in structure to the normal enzyme substrate competes with the substrate for the active site on the enzyme



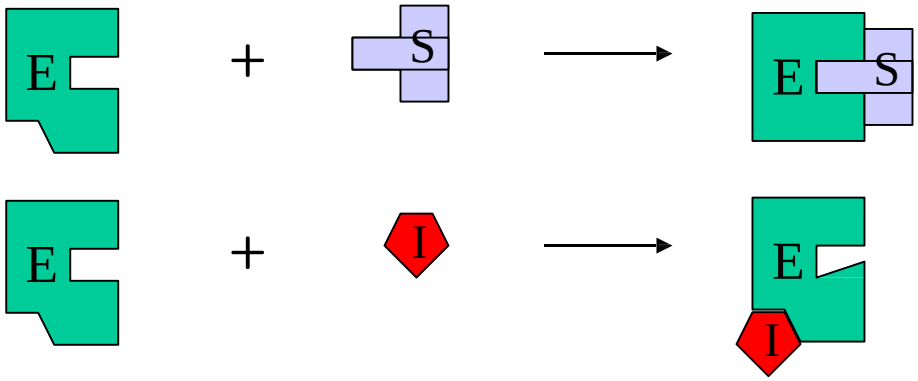
$$v = v_m \frac{S}{K_M (1 + \frac{I}{K_I}) + S} \qquad [1.16]$$

***I* : concentration of inhibitor**
K_I : competitive inhibition coefficient

1.8 Enzymes – Enzyme Reactivity

- **Noncompetitive inhibition**

-The chemical agent acts by complexing a metallic activator or binding at a place on the enzyme other than the active site. The enzyme is then less active toward its substrate



$$v = v_m \frac{S}{K_M + S} \cdot \frac{1}{1 + \frac{I}{K_I}} \qquad [1.17]$$

I **: concentration of inhibitor**
K $_I$ **: competitive inhibition coefficient**

Homework

Derive the equations below (1.16 & 1.17) based on the enzyme reactions including those of the inhibitor

$$v = v_m \frac{S}{K_M (1 + \frac{I}{K_I}) + S} \qquad [1.16]$$

$$v = v_m \frac{S}{K_M + S} \cdot \frac{1}{1 + \frac{I}{K_I}} \qquad [1.17]$$

And K_I = ?

1.9 Energy Capture

√ Electron and Energy Carriers

- All living organisms, including the microorganisms, capture energy released from oxidation-reduction reactions.
- Electrons are transferred from primary e-donor to the final e-acceptor via e- carriers (e.g., NAD^+/NADH, $NADP^+$/NADPH).
- The transfer steps have a free-energy release that the cells capture in the form of energy carriers (ATP-ADP).

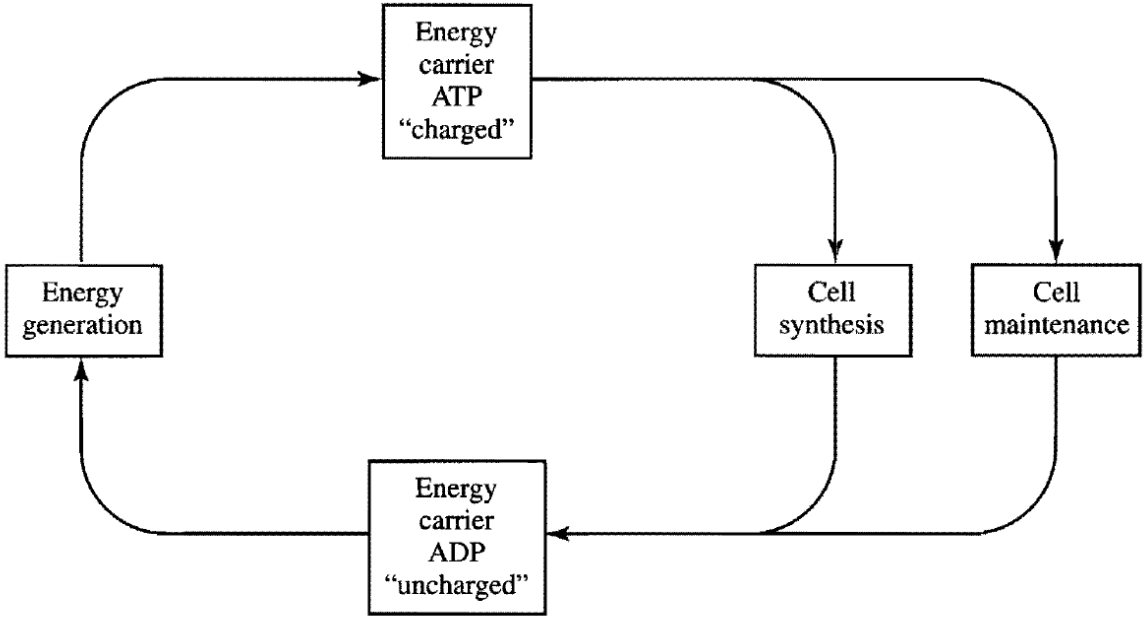


Figure 1.13 Transfer of energy from energy generation to cell synthesis or maintenance via an energy carrier, represented by ATP.

1.10 Metabolism

√ **Metabolism : The total sum of all the chemical processes of the cell**

- **Catabolism (이화작용): energy-generating reactions**

  - All the processes involved in oxidation of substrates or use of sunlight in order to obtain energy

  - It provides the energy required for 1) anabolism, 2) motion, and 3) any other energy-requiring processes

- **Anabolism (동화작용): biosynthetic reactions**

  - All the processes for the synthesis of cellular components from carbon sources

- **Metabolites : Intermediates that form in metabolism**

- **Energy coupling : transfer of energy between catabolism and anabolism**

1.10 Metabolism

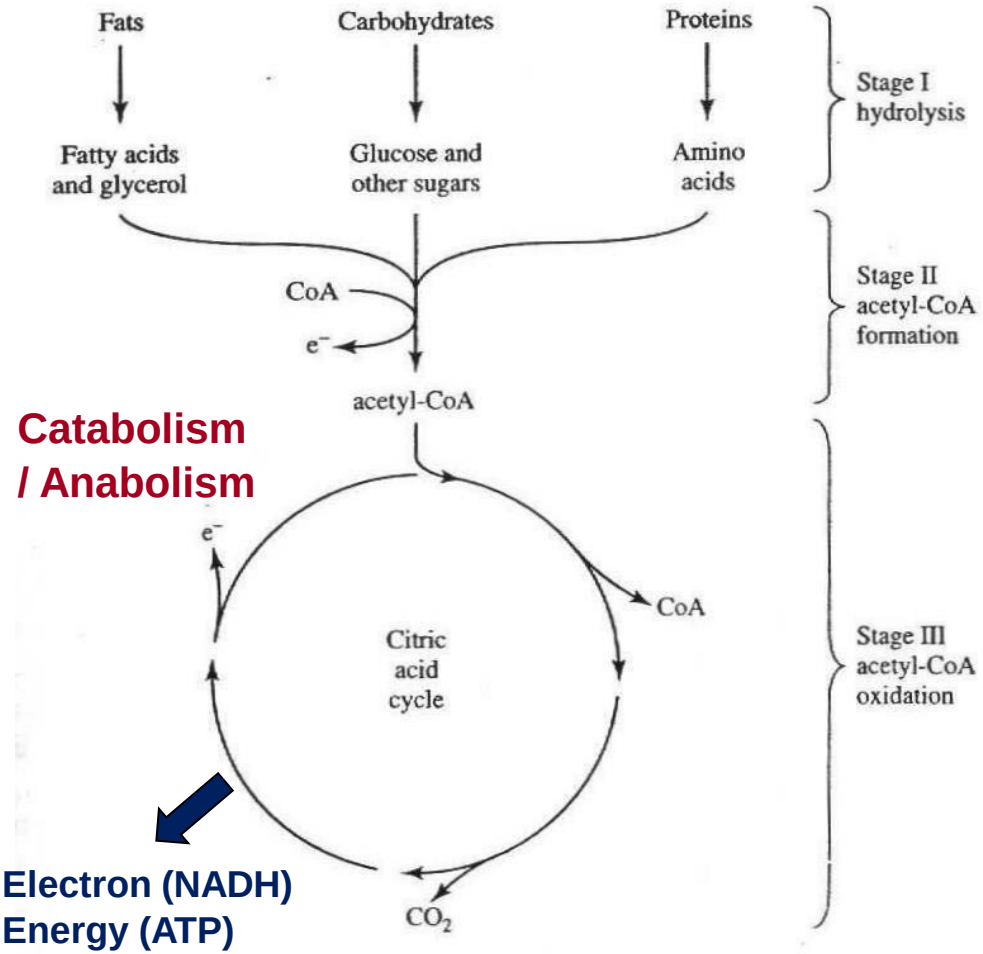


Figure 1.14 The three general stages of catabolism of fats, carbohydrates, and proteins under aerobic conditions. Reversing the processes gives anabolism.

Stage I

Degradation of large or complex molecules

Stage II

Converted into a smaller number of simpler compounds

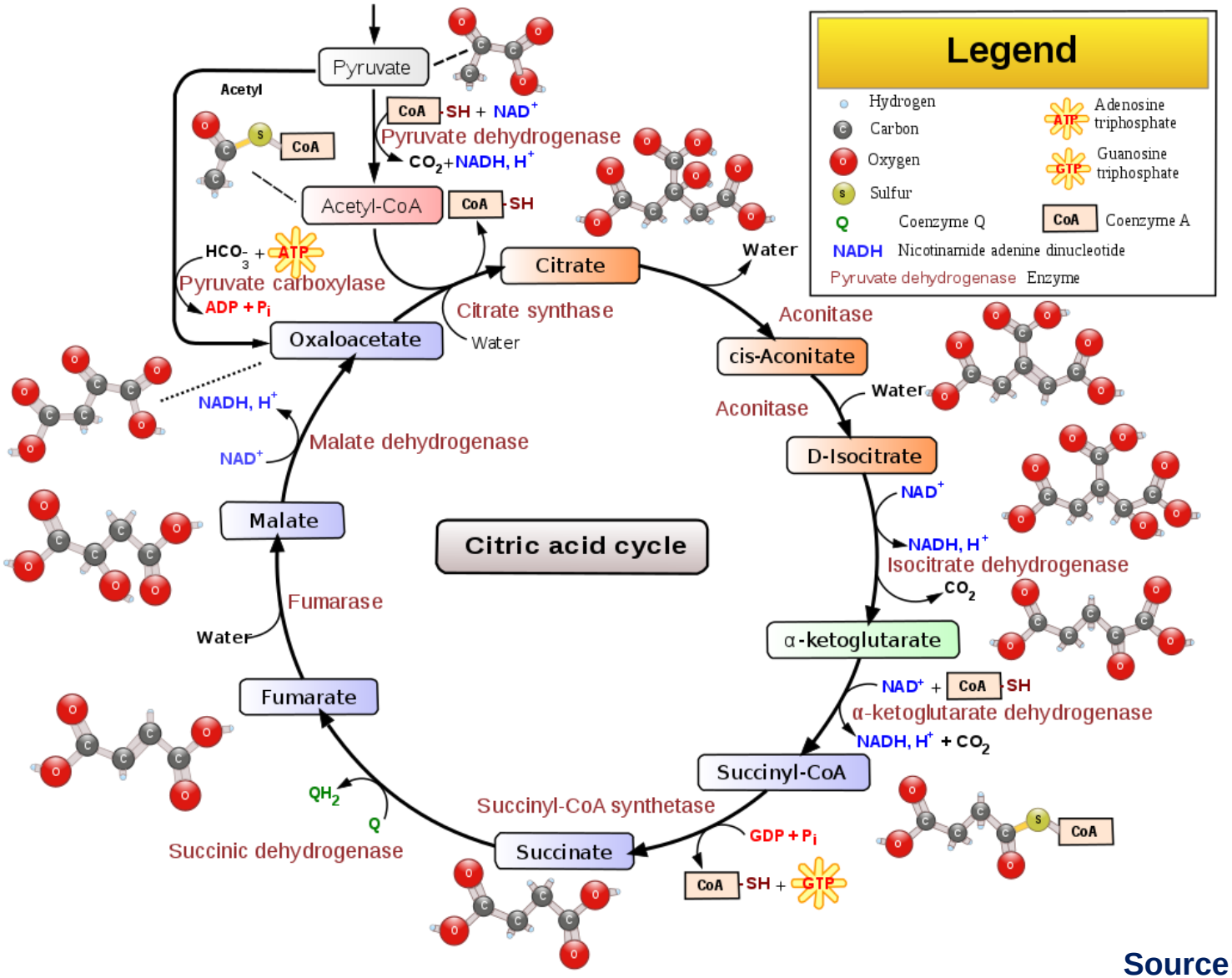
★ **fatty acids and amino acids → acetyl-CoA**

★ **hexose and pentose sugars and glycerol → glyceraldehyde-3-phosphate and pyruvate → acetyl-CoA**

Stage III

Ultimately Oxidized to CO_2 and H_2O
"the citric acid cycle" or "Krebs cycle"
Stage III generates the largest amounts of electrons and energy for the cells

1.10 Metabolism



Source: Wikipedia

1.10 Metabolism – Catabolism

√ Catabolism in chemotrophs depends on redox reactions

- **Oxidation removes electrons, and reduction adds electrons**
- **Electron donors**
  - materials that are oxidized
  - considered to be the energy substrate or "food"
  - compounds containing carbon in a reduced state : organic chemicals
  - compounds containing other elements in a reduced state : reduced inorganic compounds, such as ammonia, hydrogen, or sulfide
- **Electron acceptors**
  - materials that are reduced
  - include primarily oxygen, nitrate, nitrite, Fe(III), sulfate, and carbon dioxide
  - chlorate, perchlorate, chromate, selenite, and chlorinated organics (tetrachloroethylene, chlorobenzoate)

→ environmental concern, growing interest

1.10 Metabolism – Catabolism

√ **The quantity of energy** : **depends upon the chemical properties of the electron donor and acceptor**

Acetate and Oxygen (Aerobic Oxidation of Acetate)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{8}\,CH_3COO^- + \frac{3}{8}\,H_2O = \frac{1}{8}\,CO_2 + \frac{1}{8}\,HCO_3^- + H^+ + e^-$	− 27.40
Acceptor:	$\frac{1}{4}\,O_2 + H^+ + e^- = \frac{1}{2}\,H_2O$	− 78.72
Net:	$\frac{1}{8}\,CH_3COO^- + \frac{1}{4}\,O_2 = \frac{1}{8}\,CO_2 + \frac{1}{8}\,HCO_3^- + \frac{1}{8}\,H_2O$	− 106.12

△$G^{0\prime}$: the free energy released under standard condition (at pH 7)
= -106.12 kJ/e^- eq.

1.10 Metabolism – Catabolism

- **Methanogenesis, an anaerobic process, provides much less energy per electron equivalent than the aerobic oxidation does.**
- **Glucose contains more energy than acetate**

Acetate and Carbon Dioxide (Methanogenesis of Acetate)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{8}\,CH_3COO^- + \frac{3}{8}\,H_2O = \frac{1}{8}\,CO_2 + \frac{1}{8}\,HCO_3^- + H^+ + e^-$	− 27.40
Acceptor:	$\frac{1}{8}\,CO_2 + H^+ + e^- = \frac{1}{8}\,CH_4 + \frac{1}{4}\,H_2O$	23.53
Net:	$\frac{1}{8}\,CH_3COO^- + \frac{1}{8}\,H_2O = \frac{1}{8}\,CH_4 + \frac{1}{8}\,HCO_3^-$	− 3.87

Glucose and Carbon Dioxide (Methanogenesis from Glucose)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{24}\,C_6H_{12}O_6 + \frac{1}{4}\,H_20 = \frac{1}{4}\,CO_2 + H^+ + e^-$	− 41.35
Acceptor:	$\frac{1}{8}\,CO_2 + H^+ + e^- = \frac{1}{8}\,CH_4 + \frac{1}{4}\,H_2O$	23.53
Net:	$\frac{1}{24}\,C_6H_{12}O_6 = \frac{1}{8}\,CH_4 + \frac{1}{8}\,CO_2$	− 17.82

1.10 Metabolism – Catabolism

- **The chemolithotrophic oxidation of hydrogen produces more energy than chemoorganotrophic oxidation of acetate, but less than that of glucose**

-27.40 - 78.72 = -106.12 kJ/e- eq for acetate and oxygen

- 41.35 - 78.72 = -120.07 kJ/e- eq for glucose and oxygen

Hydrogen and Oxygen (Aerobic Oxidation of Hydrogen)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{2} H_2 = H^+ + e^-$	− 39.87
Acceptor:	$\frac{1}{4} O_2 + H^+ + e^- = \frac{1}{2} H_2O$	− 78.72
Net:	$\frac{1}{2} H_2 + \frac{1}{4} O_2 = \frac{1}{2} H_2O$	− 118.59

1.10 Metabolism – Catabolism

√ **Relative free energy available from oxidation/reduction couples**

- **"Fermentation"**

  - Use of organic compounds as electron donors and acceptors

  e.g., facultative or anaerobic bacteria

  - glucose (reactant) – ethanol (product)

  - glucose (reactant) – acetate (product)

- **Objective of catabolic reaction**

  - To capture as much of the energy released as possible

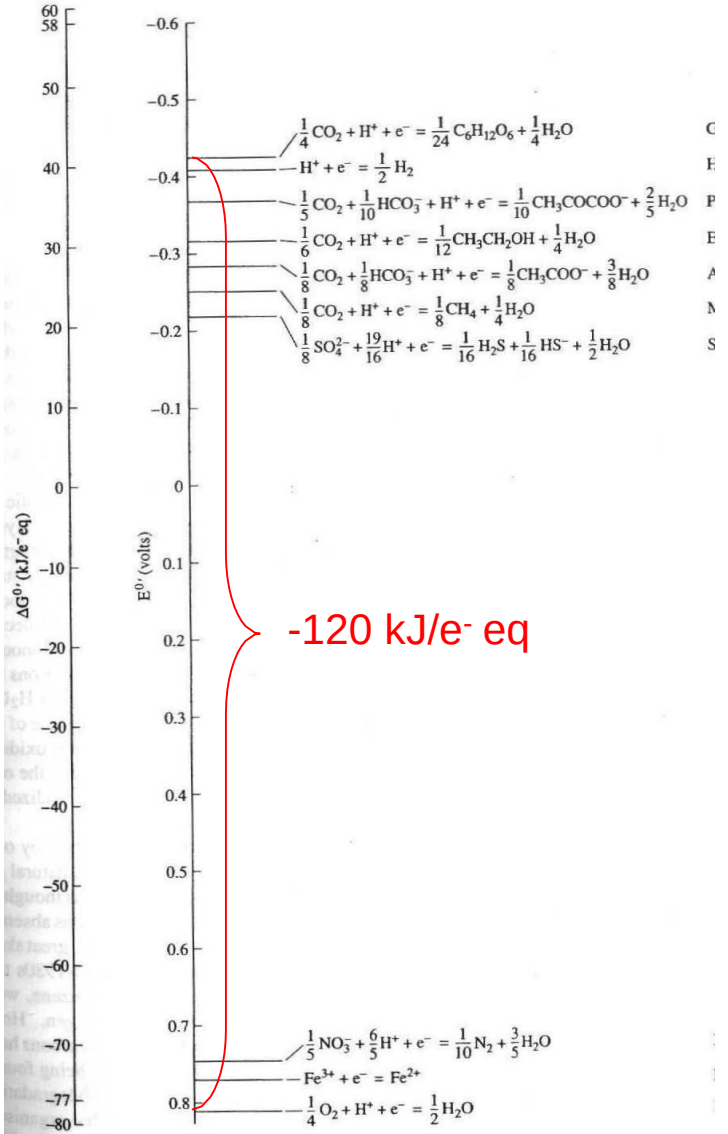


Figure 1.15 Energy scale for various oxidation/reduction couples. Energy scales can be kJ/e⁻ eq or volts, where the relationship between the two is given as one –96.485 kJ.