ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

ΑΝΟΙΚΤΑ ακαδημαϊκά μαθήματα ΠΠ

Περιβαλλοντική Βιοτεχνολογία-Environmental Biotechnology

Ενότητα 1: Basics of Microbiology

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Definition - applications

Environmental biotechnology applies the principles of microbiology to the solution of environmental problems. Applications include :

- Treatment of industrial and municipal wastewaters.
- Enhancement of the quality of drinking water.
- Restoration of industrial, commercial, residential and government sites contaminated with hazardous materials.
- Protection or restoration of rivers, lakes, estuaries and coastal waters from environmental contaminants.
- Prevention of the spread through water or air of pathogens among humans and other species.
- Production of environmentally benign chemicals.
- Reduction in industrial residuals in order to reduce resource consumption and the production of pollutants requiring disposal.

Differences between biotechnologies

The major difference between environmental biotechnology and other disciplines that feature biotechnology is that environmental applications almost always are concerned with **mixed cultures** and **open**, **nonsterile systems**. Success depends on how individual microorganisms with desired characteristics can survive in competition with other organisms, how desired functions can be maintained in complex ecosystems, and how the survival and proliferation of undesired microorganisms can be prevented.

The CELL

The cell is the basic structural and functional unit of all known **living organisms**. It is the smallest unit of an organism that is classified as living, and is often called the **building block of life**.

General principles

Each cell is at least somewhat **self-contained** and **self-maintaining:** it can take in **nutrients** ⇨ convert these nutrients into **energy** ⇨ carry out specialized **functions** ⇨ **reproduce** as necessary.

Each cell stores its own set of instructions for carrying out each of these activities.

The CELL (General principles…)

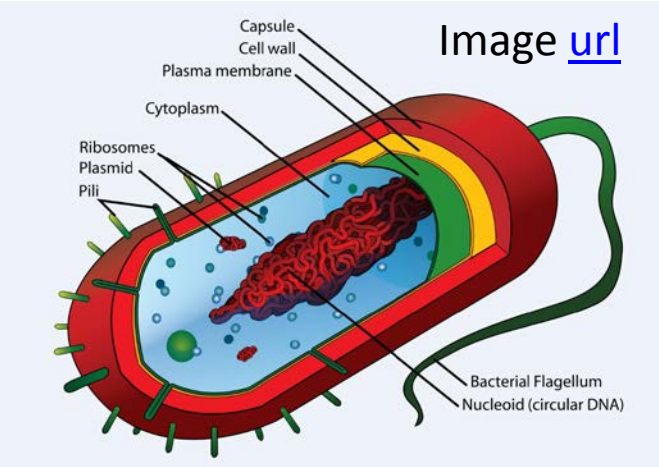
All cells have several different abilities:

- Reproduction by **cell division** : (binary fission/mitosis or meiosis).
- Use of **enzymes** and other proteins coded for by **DNA genes** and made via **messenger RNA** intermediates and **ribosomes**.
- **Metabolism**, including taking in raw materials, building cell components, converting **energy**, **molecules** and releasing **by-products**. The functioning of a cell depends upon its ability to extract and use chemical energy stored in organic molecules. This energy is released and then used in metabolic pathways.
- Response to external and internal **stimuli** such as changes in **temperature**, **pH** or **levels of nutrients**.

Cell contents are contained within a **cell surface membrane** that is made from a **lipid bilayer** with proteins embedded in it.

The CELL (Anatomy of cells)

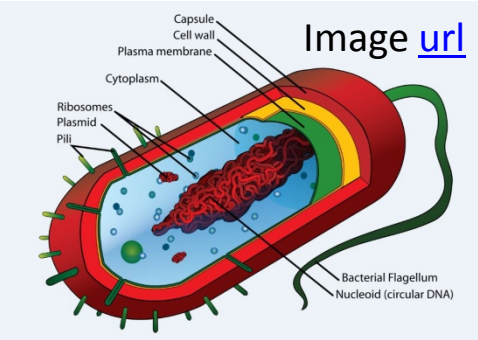
There are two types of cells: **eukaryotic** and **prokaryotic**.
Prokaryotic cells are usually independent, while eukaryotic cells are often found in multicellular organisms.



The prokaryote cell is simpler than an eukaryote cell, lacking a **nucleus** and most of the other **organelles** of eukaryotes. There are two kinds of prokaryotes: **bacteria** and **archaea**; these share a similar overall structure.

Prokaryotic cells

The CELL (Anatomy of cells)



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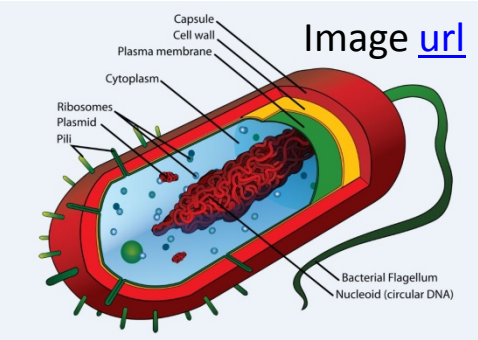
A prokaryotic cell has three architectural regions:

- on the outside, **flagella** and **pili** project from the cell's surface. These are structures (not present in all prokaryotes) made of proteins that facilitate **movement** and **communication** between cells;

- enclosing the cell is the **cell envelope** – generally consisting of a **cell wall** covering a **plasma membrane** though some bacteria also have a further covering layer called a **capsule**. The envelope gives rigidity to the cell and separates the interior of the cell from its environment, serving as a protective filter.
Though most prokaryotes have a cell wall, there are exceptions such as **Mycoplasma** (bacteria) and **Thermoplasma** (archaea)). The cell wall consists of **peptidoglycan** in bacteria, and acts as an additional barrier against exterior forces. It also prevents the cell from expanding and finally bursting (**cytolysis**) from **osmotic pressure** against a **hypotonic** environment. Some eukaryote cells (**plant cells** and **fungi** cells) also have a cell wall;

The CELL (Anatomy of cells)



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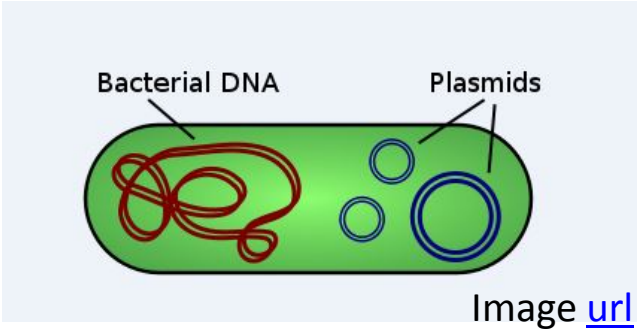
- Inside the cell is the **cytoplasmic region** that contains water and macromolecules like the **cell genome** (DNA) and **ribosomes** and various sorts of **inclusions**. A prokaryotic chromosome is usually a circular molecule (an exception is that of the bacterium **Borrelia burgdorferi**, which causes **Lyme disease**). Though not forming a **nucleus**, the DNA is condensed in a **nucleoid**.

Prokaryotes can carry **extrachromosomal DNA** elements called **plasmids**, which are usually circular. Plasmids enable additional functions, such as **antibiotic resistance**.



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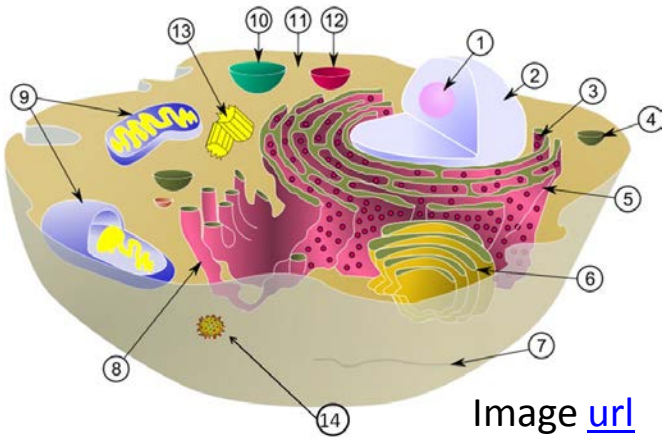
Chromosome ⇨ **ribosome** ⇨ **enzymes**
genetic code — converting engine — catalyst

The CELL (Anatomy of cells)

Eukariotic cells are about 10 times the size of a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is the presence of a **cell nucleus**, a membrane-delineated compartment that houses the eukaryotic cell's DNA. It is this nucleus that gives the eukaryote its name, which means **"true nucleus“**.



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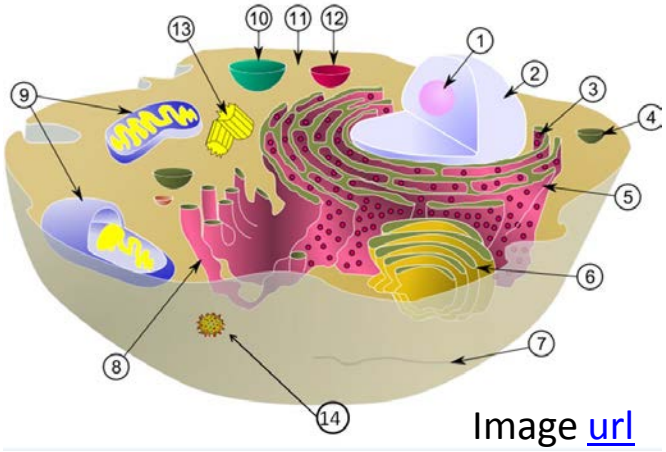
Eukaryotic cells

Organelles:

(1) Nucleolus
(2) Nucleus
(3) Ribosome
(4) Vesicle
(5) Rough endoplasmic reticulum
(6) Golgi apparatus
(7) Cytoskeleton
(8) Smooth endoplasmic reticulum
(9) Mitochondria
(10) Vacuole
(11) Cytoplasm
(12) Lysosome
(13) Centrioles

The CELL (Anatomy of cells)



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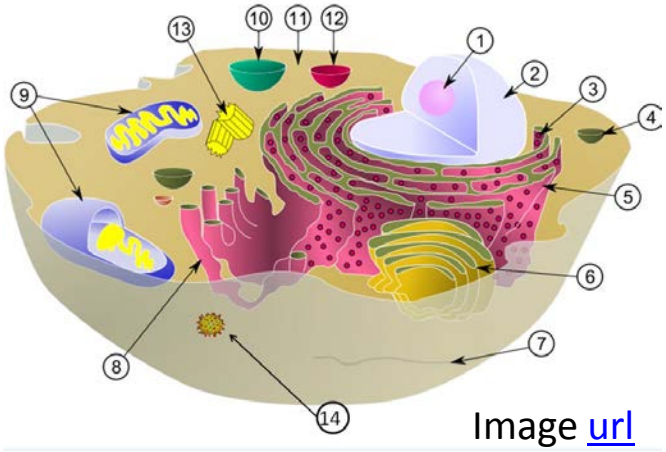
Eukaryotic cells

Other differences include:

The plasma membrane resembles that of prokaryotes in function, with minor differences in the setup. Cell walls may or may not be present.

The eukaryotic DNA is organized in one or more linear molecules, called **chromosomes**, which are associated with **histone** proteins. All chromosomal DNA is stored in the **cell nucleus**, separated from the cytoplasm by a membrane. Some eukaryotic **organelles** such as **mitochondria** also contain some **DNA**.

The CELL (Anatomy of cells)



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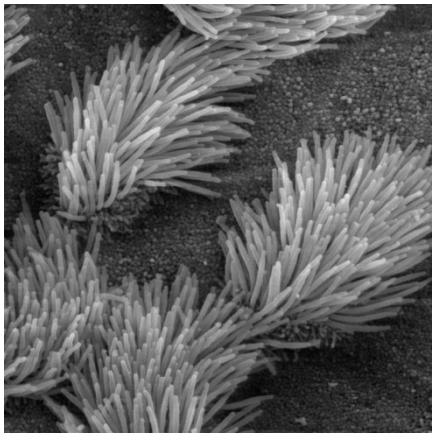
Eukaryotic cells

Many eukaryotic cells are **ciliated** with **primary cilia**.

Primary cilia play important roles in **chemosensation**, **mechanosensation**, and **thermosensation**.

Cilia may thus be "**viewed as sensory cellular antennae** that coordinate a large number of cellular signaling pathways, sometimes coupling the signaling to ciliary motility or alternatively to cell division and differentiation."
Eukaryotes can move using **motile cilia** or **flagella**. The flagella are more complex than those of prokaryotes.



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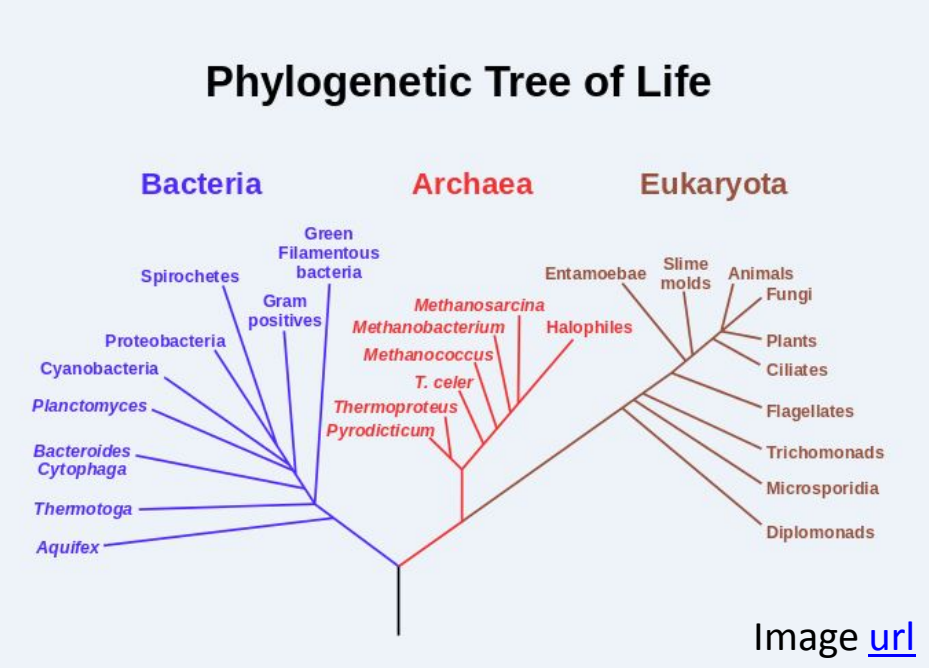
The CELL (phylogenetic tree)



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A **phylogenetic tree** or **evolutionary tree** is a tree showing the evolutionary relationships among various biological species or other entities that are believed to have a common ancestor. In a phylogenetic tree, each node with descendants represents the most recent common ancestor of the descendants, and the edge lengths in some trees correspond to time estimates.

Each node is called a **taxonomic unit**. Internal nodes are generally called hypothetical taxonomic units (HTUs) as they cannot be directly observed.

The CELL (Taxonomy and Phylogeny)

Taxonomy is the science of classification of microorganisms and relies on the observable physical properties of organisms.

Observable properties are called cell's **phenotype** and may involve its appearance (**morphology**), the manner in which it interacts with dyes or staining and its ability to use or convert a given chemical into another one (**transformation**).

Phylogeny is a newer method of classification that detects differences in microorganisms based upon genetic characteristics which are encoded in the organism's DNA and RNA.

The basic taxonomic unit is the **species** which is the collection of strains having sufficiently similar characteristics. Groups of species with major similarities are placed in collections called **genera** and groups of genera with sufficient similarities are collected into **families**. Microorganisms are generally given a genus and species name. For example : Escherichia coli or E.coli.

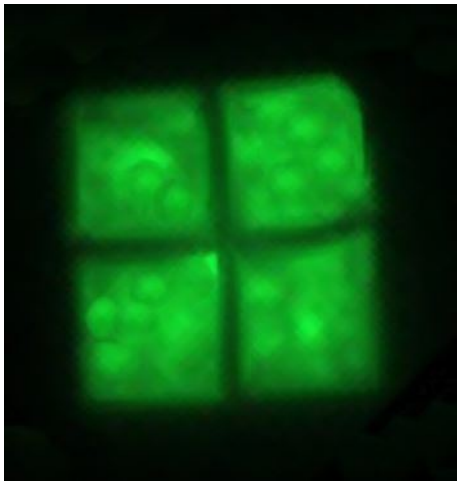
Archaea

The **Archaea** are a group of single-celled microorganisms. They have nocell nucleus or any other **organelles** within their cells.

Initially, archaea were seen as **extremophiles** that lived in harsh environments, such as **hot springs** and **salt lakes**, but they have since been found in a broad range of habitats, such as soils, oceans and marshlands. Archaea are particularly numerous in the oceans, and the archaea in plankton may be one of the most abundant groups of organisms on the planet. Archaea are now recognized as a major part of life on Earth and may play an important role in both the **carbon cycle** and **nitrogen cycle**. No clear examples of archaeal pathogens or parasites are known, but they are often **mutualists** or **commensals**.

Archaea

One example are the **methanogenic archaea** that inhabit the gut of humans and ruminants, where they are present in vast numbers and aid in the digestion of food. Archaea have some importance in technology, with methanogens used to produce biogasand as part of sewage treatment and enzymes from extremophile archaea that can resist high temperatures and organic solvents are exploited in **biotechnology**.



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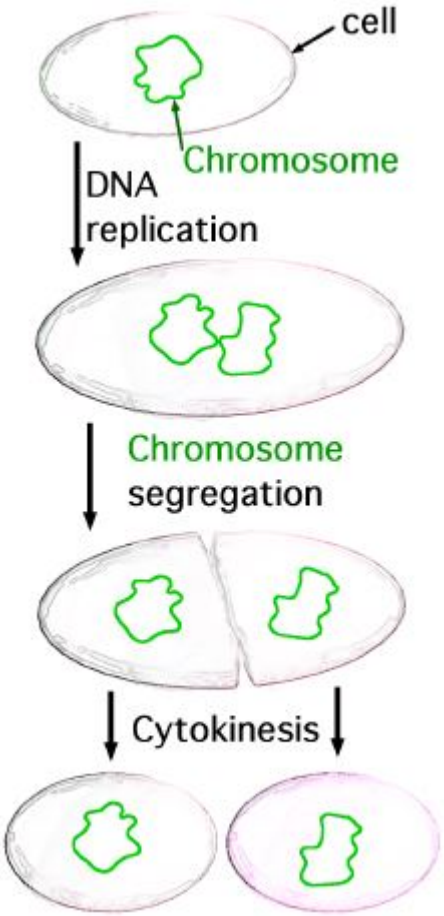
Generally, archaea and bacteria are quite similar in **size** and **shape**, although a few archaea have very unusual shapes, such as the flat and square-shaped cells of **Haloquadratum walsbyi**.

Despite this visual similarity to bacteria, archaea possess genes and several metabolic pathways that are more closely related to those of eukaryotes: notably the enzymes involved in **transcription** and **translation**.

Archaea

Other aspects of archaean biochemistry are unique, such as their reliance on **ether lipids** in their **cell membranes**. The archaea exploit a much greater variety of sources of energy than eukaryotes: ranging from familiar organic compounds such as **sugars**, to using **ammonia**, **metal ions** or even **hydrogen gas** as nutrients.

Salt-tolerant archaea (the **Halobacteria**) use sunlight as a source of energy, and other species of archaea **fix carbon**; however, unlike plants and cyanobacteria, no species of archaea is known to do both. Archaea reproduce **asexually** and divide by **binary fission**, **fragmentation**, or **budding**; in contrast to bacteria and eukaryotes, no species of archaea are known that form **spores**.



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Archaea

Morphology

Individual archaeans range from **0.1 µm** to over **15 µm** in diameter, and occur in various shapes, commonly as **spheres**, **rods**, **spirals** or **plates**. Other morphologies in the Crenarchaeota include irregularly shaped lobed cells in Sulfolobus, thin needle-like filaments that are less than half a micrometer in diameter in Thermofilum, and almost perfectly rectangular rods in Thermoproteus and Pyrobaculum.

Archaea

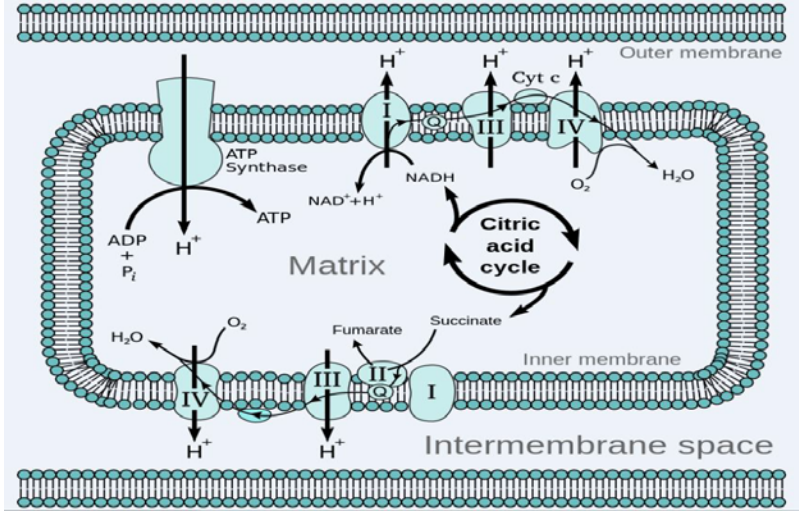
Metabolism

Archaea exhibit a great variety of chemical reactions in their metabolism and use many different sources of energy. These forms of metabolism are classified into nutritional groups, depending on the **source of energy** and the **source of carbon**. Some archaea obtain their energy from inorganic compounds such as **sulfur** or **ammonia** (they are **lithotrophs**). These archaea include **nitrifiers**, **methanogens** and **anaerobic methane oxidizers**.

In these reactions **one compound passes electrons to another (in a redox reaction), releasing energy that is then used to fuel the cell's activities.** One compound acts as an **electron donor** and one as an **electron acceptor**. A common feature of all these reactions is that the energy released is used to generate adenosine triphosphate (ATP) through **chemiosmosis**, which is the same basic process that happens in the mitochondrion of animal cells.

Archaea

Chemiosmosis is the diffusion of ions across a selectively-permeable membrane. More specifically, it relates to the generation of ATP by the movement of hydrogen ions across a membrane during cellular respiration.



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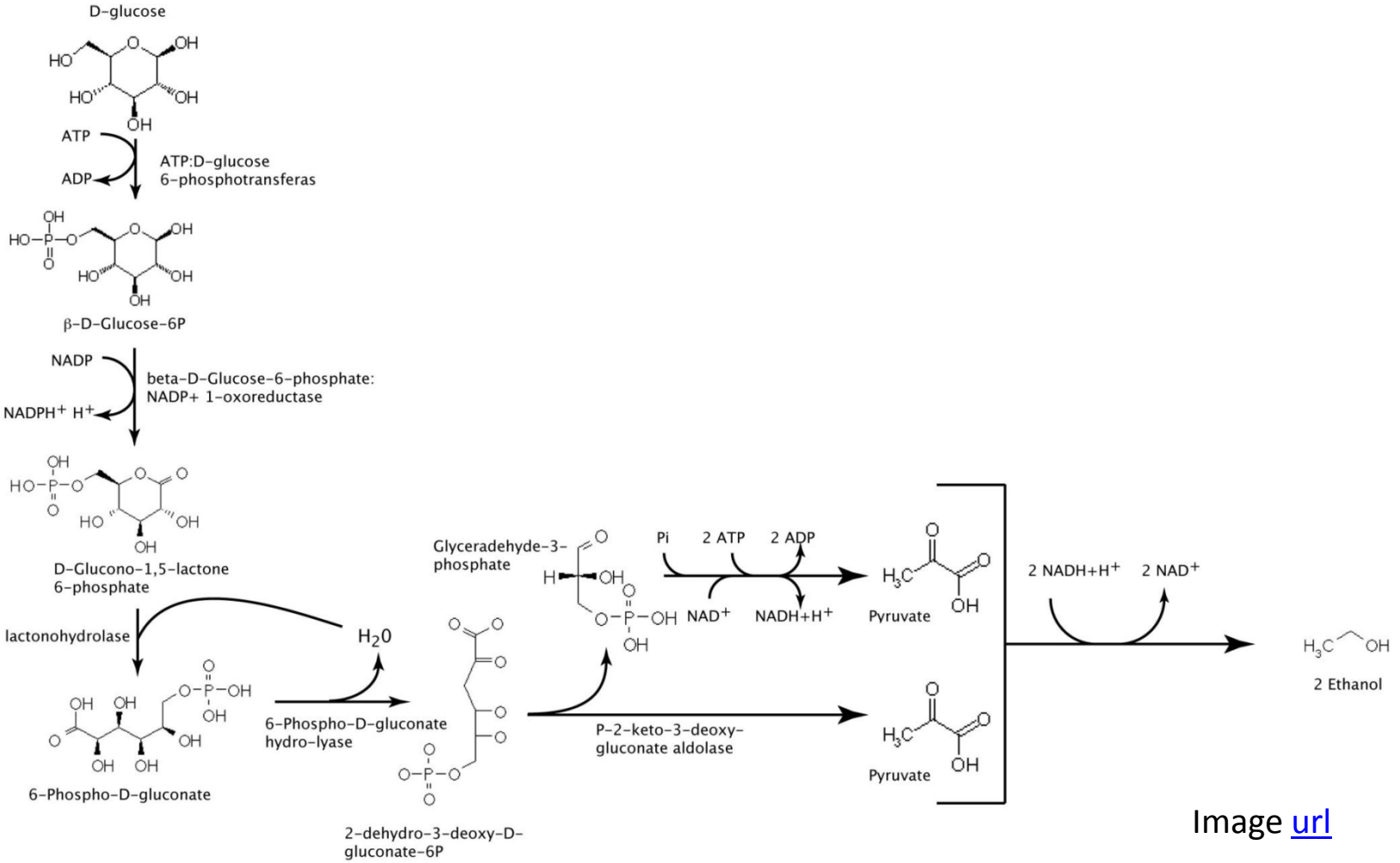
Other groups of archaea use sunlight as a source of energy (they are **phototrophs**). However, oxygen-generating photosynthesis does not occur in any of these organisms. Archaea use a modified form of **glycolysis** (the **Entner-Doudoroff pathway**) and either a complete or partial **citric acid cycle**.

Archaea

Nutritional types in archaeal metabolism.

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds	Halobacteria
Lithotrophs	Inorganic compounds	Organic compounds or carbon fixation	Ferroglobus, Methanobacteria or Pyrolobus
Organotrophs	Organic compounds	Organic compounds or carbon fixation	Pyrococcus, Sulfolobus or Methanosarcinales

Entner-Doudoroff pathway



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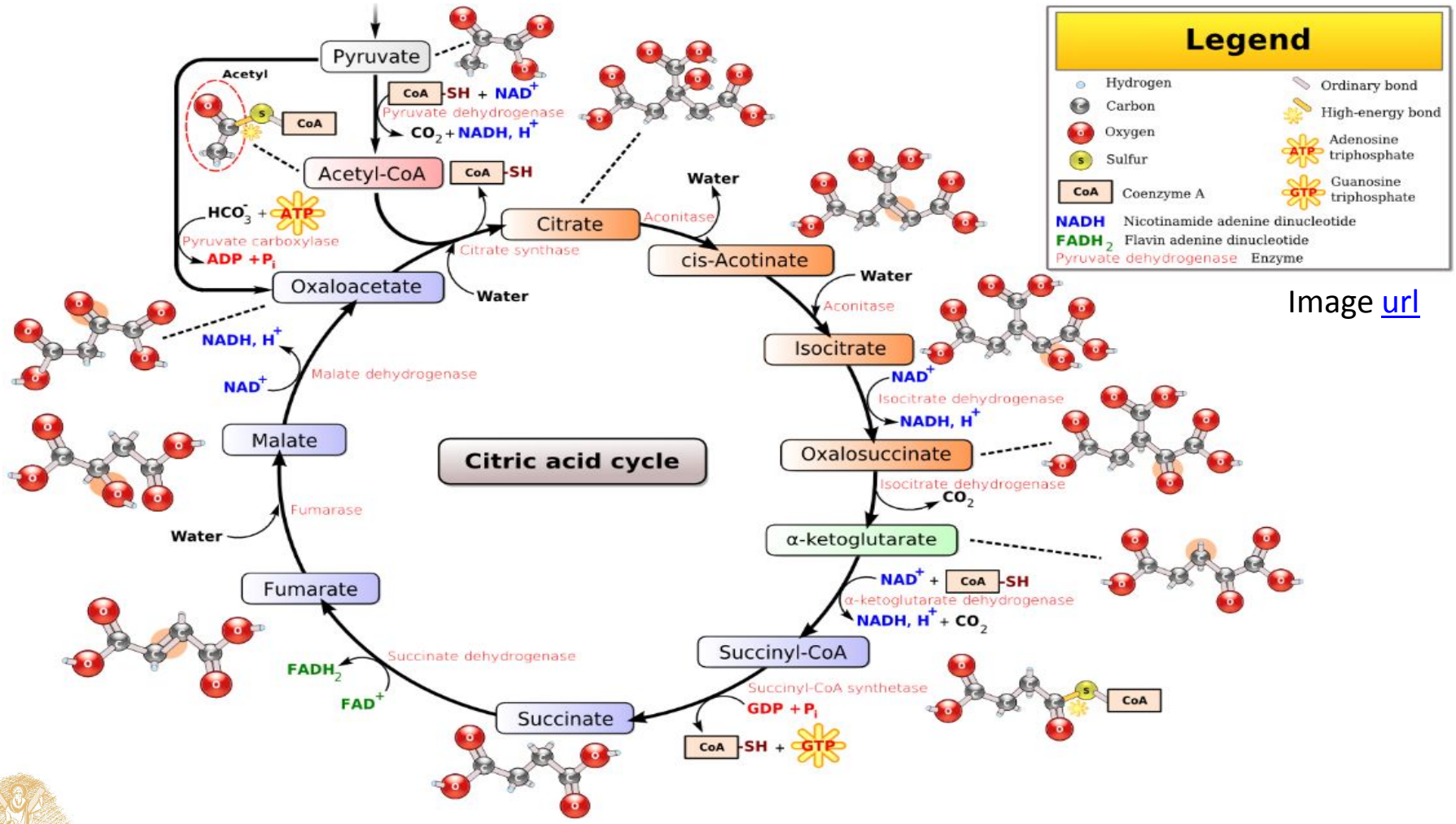
Citric acid cycle



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Bacteria

Bacteria are among the smallest living entities.

They are present in **land**, **water** and **air**.

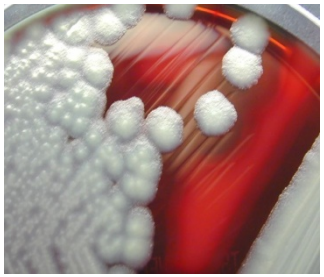
They are important to the environment, as they:

- have the ability to **transform** organic & inorganic pollutants into harmless minerals recycled back to the environment
- **convert** inorganic nitrogen to N_2
- **degrade** organic chemicals and industrially synthesized compounds,
- **produce** methane, hydrogen and other bio-fuels.

Bacteria

Morphology

Morphology includes **shape**, **size**, **structure** and **spatial relationship** to one another.



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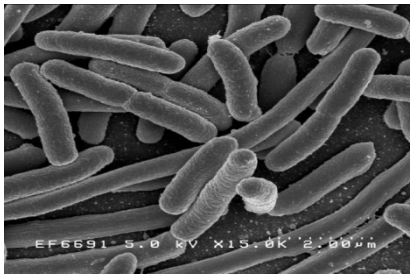
Bacillus cereus



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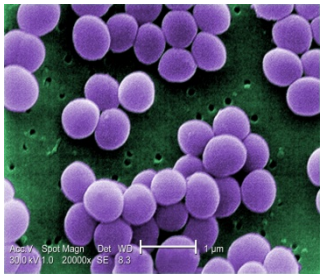
Escherichia coli (rods or bacilli)



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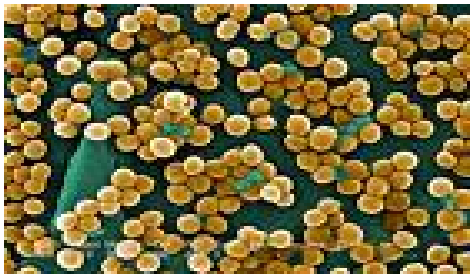

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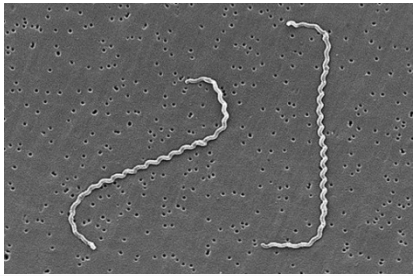
Staphylococcus aureus (cocci)



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Leptospira interrogans (spirillum)

Bacteria

Morphology

Width varies from 0.5 to 2 µm and **Length** from 1 to 5 µm.

Cocci diameter within 0.5 to 5 µm range. About 10^{12} bacteria per g d.w.

Surface about 12 m^2/g d.w.

Cytoplasmic inclusions may include polyphosphate granules, PHB or fatty materials. Sulfur accumulates in sulfur-metabolizing bacteria.

Bacterial cell walls

The plasma membrane of Gram-negative bacteria is surrounded by a thin cell wall beneath the outer membrane.

Gram-positive bacteria lack outer membranes and have thick cell walls.

Bacterial cells (chemical composition)

In order for bacteria to grow and maintain themselves they must have essential nutrients such as **C, N, P, S** and other **trace elements**.

Trace elements

Mo for N_2 fixation

Ni for anaerobic CH_4 prod.

Cofactors such as vitamins

Empirical formula

$C_5H_7O_2N$

MW = 113

C : 53% MW

N : 12,4% MW

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

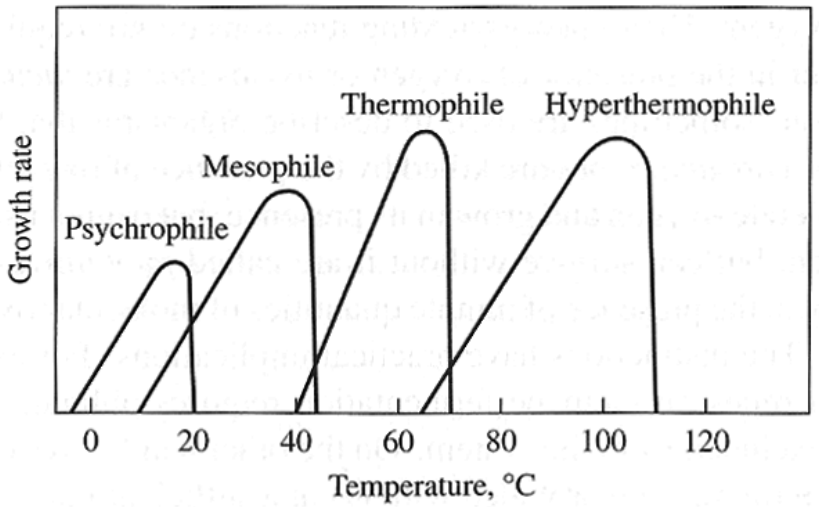
Bacterial cells (environmental conditions)

Factors of importance : **Temperature**, **pH**, **DO** and **osmotic pressure**.

Based on D.O.
Aerobic vs Anaerobic
Facultative bacteria
Obligate vs aerotolerant anaerobs
Microaerophiles

Based on salt conc.
Halophiles (~3,5% NaCl)
Extreme halophiles (15-30%)

Temperature Class	Normal Temperature Range for Growth (°C)
Psychrophile	−5 to 20
Mesophile	8 to 45
Thermophile	40 to 70
Hyperthermophile	65 to 110



Bacteria

Characteristics of the 12 phylogenic lineages of bacteria

Phylogenic Group	**Characteristics**
Aquifex/Hydrogenobacter	Hyperthermophilic, chemolithotrophic
Thermotoga	Hyperthermophilic, chemoorganotrophic, fermentative
Green nonsulfer bacteria	Thermophilic, phototrophic and nonphototrophic
Deinococci	Some thermophiles, some radiation resistant, some unique spirochetes
Spirochetes	Unique spiral morphology
Green sulfur bacteria	Strictly anaerobic, obligately anoxygenic phototrophic
Bacteroides-Flavobacteria	Mixture of types, strict aerobes to strict anaerobes, some are gliding bacteria
Planctomyces	Some reproduce by budding and lack peptidoglycan in cell walls, aerobic, aquatic, require dilute media
Chlamydiae	Obligately intracellular parasites, many cause diseases in humans and other anomals
Gram-positive bacteria	Gram-positive, many different types, unique cell-wall composition
Cyanobacteria	Oxygenic phototrophic
Purple bacteria	Gram-negative; many different types including anoxygenic phototrophs and nonphototrophs; aerobic, anaerobic and facultative; chemoorganotrophic and chemolithotrophic

Bacteria

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
Epsilon	Thiovulum, Wolinella, Campylobacter, Helicobacter

*Phototrophic representatives

Enzymes

Organic catalysts produced by m/o to speed up **energy-yielding** and **cell-building** reactions within the cells.

Primary structure → chain of attached amino acids.

Secondary structure → twisted chain of amino acids into a 3-dimensional configuration (amino acid and S-S bonding)

Tertiary structure → protein folded back onto itself (H-H bonding)

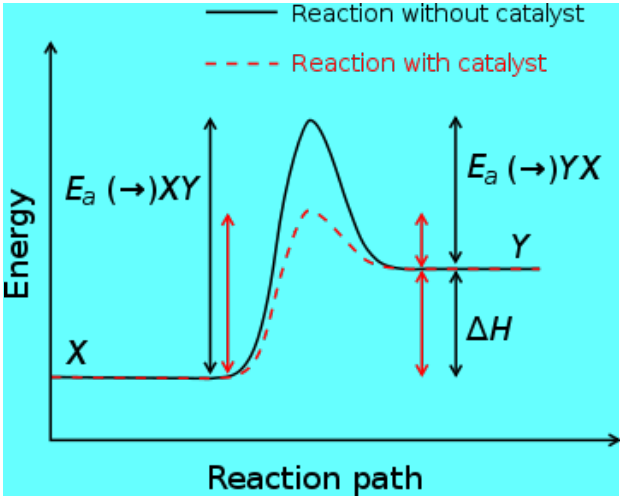
Denaturation : breaking down of tertiary structure by heat, pH and chemicals

Important characteristics of enzymes : **specificity** and **rate of reaction**

The set of enzymes made in a cell determines which **metabolic pathways** occur in that cell

Enzymes

- Enzymes **do not increase** the amount of energy released by a given reaction but they **minimize the diversion of resources** (electrons, energy and elements) into non-productive pathways.
- A single enzyme molecule can affect from 1.000-100.000 molecular reactions/sec.
- Enzymes **are not consumed** by the reactions they catalyze, nor do they alter the **equilibrium** of these reactions.



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A catalyst increases the rate of reaction without being consumed by it. In addition, while the catalyst lowers the activation energy, it does not change the energies of the original reactants nor products. Rather, the reactant energy and the product energy remain the same and only the **activation energy** is altered (lowered).

Enzymes

Activity of enzymes

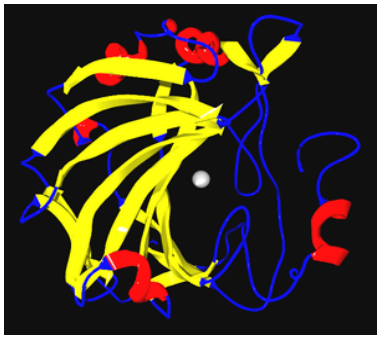
a. Their activity may depend only on their structure

b. Others require a non-protein component.

1. Metal ion → cofactor

2. Organic → coenzyme or prosthetic group.

Carbonic anhydrase II.



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Metal Cofactor	**Enzyme or Function**
Co	Transcarboxylase, Vitamin B_{12}
Cu	Cytochrome c oxidase, proteins involved in respiration, some superoxide dismutases
Fe	Activates many enzymes, catalases, oxygenases, cytochromes, nitrogenases, peroxidases
Mn	Activates many enzymes, oxygenic photosynthesis, some superoxide dismutases
Mo	Nitrate reductase, formate dehydrogenases, oxotransferases, molybdenum nitrogenase
Ni	Carbon monoxide dehydrogenase, most hydrogenases, coenzyme F_{430} of methanogens, urease
Se	Some hydrogenases, formate dehydrogenase
V	Vanadium nitrogenase, some peroxidases
W	Oxotransferases of hyperthermophiles, some formate dehydrogenase
Zn	RNA and DNA polymerase, carbonic anhydrase, alcohol dehydrogenase

Enzymes

Coenzymes involved in group-transferring reactions

Group Transferred	Coenzyme	Acronym
Hydrogen atoms (electrons)	Nicotinamide adenine dinucleotide	NAD
	Nicotinamide adenine dinucleotide phosphate	NADP
	Flavin adenine dinucleotide	FAD
	Flavin mononucleotide	FMN
	Coenzyme Q	CoQ
	Coenzyme F_{420}	F_{420}
Acyl groups	Lipoamide	
	Coenzyme A	HSCoA
One-carbon units	Tetrahydrofolate	
	Methanofuran	
	Tetrahydromethanopterin	
	Coenzyme M	CoM
Carbon dioxide	Biotin	
Methyl	S-Adenosylmethionine	
Glucose	Uridinediphosphate glucose	
Nucleotides	Nucleotide triphosphates	
Aldehyde	Thiamine pyrophosphate	

Enzymes

NAME : -ase added to a root meaning either the reaction catalyzed or the substrate transformed.

proteinase *:* hydrolyzes proteins to form amino acids

dehydrogenase *:* removes 2 hydrogen atoms from a molecule

In oxidation-reduction reactions enzymes catalyze reactions where electrons are transferred from an **electron donor** to an **electron acceptor.**

Acetate is oxidized transferring electrons to O_2 which is converted to water.

↑ **primary electron donor** ↑ **final electron acceptor**

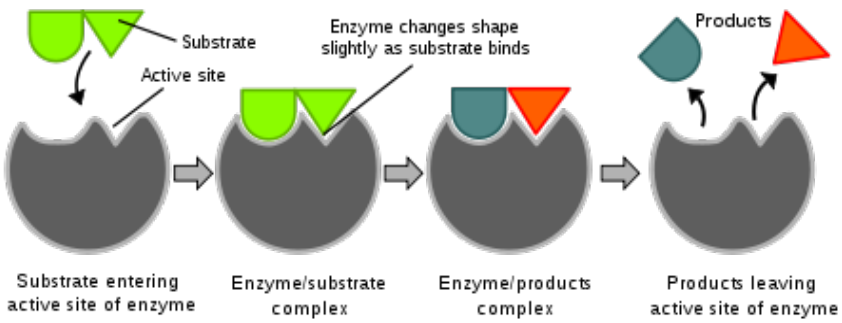
Extacellular or exoenzymes vs intracellular or endoenzymes

Enzyme Reactivity

Specificity

"Lock and key" model (Emil Fischer, 1894)
The enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. Fails to explain the stabilization of the transition state that enzymes achieve.

"Induced fit model" model (Daniel Koshland, 1958)
Since enzymes are rather flexible structures, the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme.



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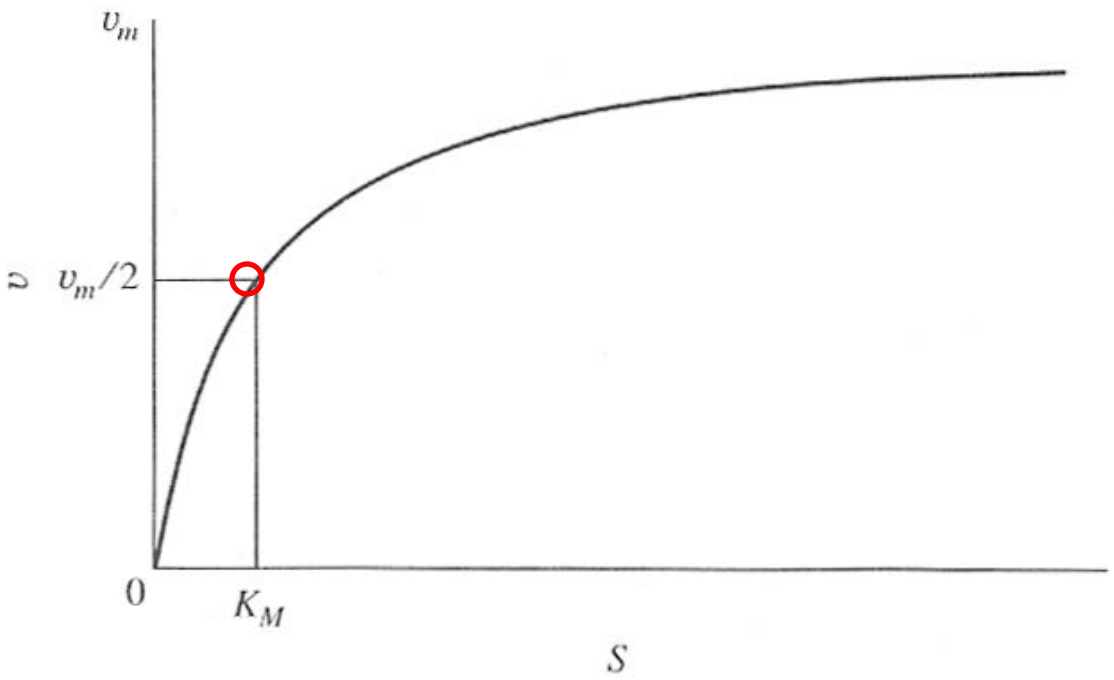
Apparently, the most effective way for reaching large stabilization is the use of **electrostatic effects**, in particular, by having a relatively fixed polar environment that is oriented toward the charge distribution of the transition state. Such an environment does not exist in the uncatalyzed reaction in water.

Enzyme Reactivity

A characteristic of enzyme reactions is **substrate saturation**.



L. Michaelis and M.L. Menten developed a theory on enzyme kinetics describing this phenomenon in 1913. G.E. Briggs and J.B.S Haldane extended this theory.

Enzyme Reactivity

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Both **reactions** are considered reversible, and the various *k* values are rate coefficients for each of the four possible reactions. In the Briggs and Haldane development, *E* equals the total enzyme concentration, *ES* is the concentration of enzyme-substrate complex and the difference between the two, *E – ES*, is the concentration of free enzyme.

The rate of formation of *ES* from *E + S* is thus given by:

$$\frac{dES}{dt} = k_1 \left(E - ES \right) S$$

The rate of formation of *ES* from *E + P* is very small and neglected. The rate of breakdown of *ES* is thus given by:

$$-\frac{dES}{dt} = k_{-1}\, ES + k_2 ES$$

Enzyme Reactivity

When the rate of formation of ES just equals its rate of breakdown, the system is at steady state with respect to ES concentration:

$$k_1\left(E-ES\right)S=k_{-1}ES+k_2ES$$

Rearranging gives:

$$\frac{S\left(E-ES\right)}{ES}=\frac{k_{-1}+k_2}{k_1}=K_M$$

The coefficient K_M, which represents a composite of the three rate coefficients, is called the **Michaelis-Menten coefficient**.

It may be solved for the concentration of the ES complex

$$ES=\frac{E\cdot S}{K_M+S}$$

Our interest is in the overall rate of the reaction, in other words, the rate of formation of product P. The velocity of this reaction υ is given by:

$$\upsilon=k_2ES$$

Combining this two Equation yields

$$\upsilon=\frac{k_2\cdot E\cdot S}{K_M+S}$$

Enzyme Reactivity

If the substrate concentration is very high so that essentially all the enzyme is present as the ES complex, that is, ES = E, then the maximum velocity, υ_m , is obtained:

$$\upsilon_m = k_2 E$$

The **Michaelis-Menten equation**, which defines the quantitative relationship between the substrate concentration and the reaction rate in relation to the maximum possible rate:

$$\upsilon = \upsilon_m \frac{S}{K_M + S}$$

For the important case where $\upsilon = \frac{1}{2}\,\upsilon_m$,

$$\frac{1}{2} = \frac{S}{K_M + S} \text{ or } \upsilon = \frac{\upsilon_m}{2} \longrightarrow S = K_M, \text{ when } \upsilon = \frac{\upsilon_m}{2}$$

Enzyme Reactivity

Effect of chemicals

Chemical agents may reduce enzyme activity.

Two types of reversible inhibition is **competitive** and **non-competitive**.

In **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. For example, trichloroethene complexed at the active site of methane monooxygenase prevents the enzyme from complexing with methane, causing the rate of methane oxidation to decrease. However, if the methane concentration is increased, its reaction rate also increases, because methane then displaces trichloroethene from the enzyme. A competitive-inhibition model, developed from fundamental principles similar to the treatment above, yields the following result:

$$\upsilon = \upsilon_m \frac{S}{K_M\left(1+\frac{I}{K_I}\right)+S}$$

Enzyme Reactivity

Effect of chemicals

In **noncompetitive inhibition**, the chemical agent acts by complexing a metallic activator or by binding at a place on the enzyme other than the active site. The enzyme is then less reactive toward its substrate. For example, cyanide affects enzymes that require iron for activation, because it forms a strong complex with this metal. Metals such as Cu(II), Hg(II), and Ag(I) combine with sulfhydryl groups (-SH) of cysteine, a common amino acid in proteins, and thus affect enzyme activity. In noncompetitive inhibition, an increase in substrate concentration does not counteract the effect of the inhibitor. The model for noncompetitive inhibition, developed from fundamental principles, is

$$\upsilon = \upsilon_m \frac{S}{K_M + S} \cdot \frac{1}{1 + \frac{I}{K_I}}$$

Electron and energy carriers

2 different classes :

a. Freely diffusible throughout the cell's cytoplasm
   (**NAD^+** and **$NADP^+$**)
b. Attached in the cytoplasmic membrane
   (**NADH dehydrogenases**, **cytochromes** and **quinones**).

The reactions of NAD^+ and $NADP^+$:

$$NAD^+ + 2H^+ + 2e^- = NADH + H^+ \qquad \Delta G^{0'} = 62kJ$$

$$NADP^+ + 2H^+ + 2e^- = NADPH + H^+ \qquad \Delta G^{0'} = 62kJ$$

Electron and energy carriers

If **oxygen** is the **terminal electron acceptor**, the energy released as the electrons are passed through a chain of electron carriers to oxygen can be determined from the overall free energy change of the NADH and O_2 half reactions:

$$NADH + H^+ = NAD^+ + 2H^+ + 2e^- \qquad \Delta G^{0'} = -62kJ$$

$$\frac{1}{2}O_2 + 2H^+ + 2e^- = H_2O \qquad \Delta G^{0'} = -157kJ$$

$$Net: NADH + \frac{1}{2}O_2 + H^+ = NAD^+ + H_2O \qquad \Delta G^{0'} = -219kJ$$

Thus, the energy transferred along with electrons from an organic chemical to NADH is released to subsequent electron carriers and ultimately to oxygen in aerobic respiration, yielding in this case -219 kJ per mole of NADH for use by the organism.

Electron and energy carriers

How is this energy captured? It is accomplished by transferring the energy from intermediate electron carriers to energy carriers. The primary example of an **energy carrier** is adenosine triphosphate (ATP). When energy is released from an electron carrier, it is used to add a phosphate group to adenosine diphosphate (ADP):

$$ADP + H_3PO_4 = ATP + H_2O \qquad \Delta G^{0'} = 32kJ$$

$$or\ in\ simplified\ \ form:$$

$$ADP + P_i = ATP + H_2O \qquad \Delta G^{0'} = 32kJ$$

Electron and energy carriers

One question of interest is how many ATPs can be formed from NADH under anaerobic conditions. This might be estimated by computing the overall free energy released when other known electron acceptors accept the electrons from NADH:

$$NO_3^- : NADH + \frac{2}{5} NO_3^- + \frac{7}{5} H^+ = NAD^+ + \frac{1}{5} N_2 + \frac{6}{5} H_2O \qquad \Delta G^{0'} = -206kJ$$

$$SO_4^{2-} : NADH + \frac{1}{4} SO_4^{2-} + \frac{11}{8} H^+ = NAD^+ + \frac{1}{8} H_2S + \frac{1}{8} HS^- + H_2O \qquad \Delta G^{0'} = -20kJ$$

$$CO_2 : NADH + \frac{1}{4} CO_2 + H^+ = NAD^+ + \frac{1}{4} CH_4 + \frac{1}{2} H_2O \qquad \Delta G^{0'} = -15kJ$$

Metabolism

The sum total of all the chemical processes of the cell.

Catabolism : all the processes involved in the oxidation of substrates in order to obtain energy or use of sun-light. Also furnishes energy for motion or other energy-requiring processes.

Anabolism : all the processes for the synthesis of cellular components from carbon sources.

Energy-yielding → metabolites ….metabolites → final end-products substrate

↓

NADH or **ATP** formation

Catabolism → → → → → Anabolism

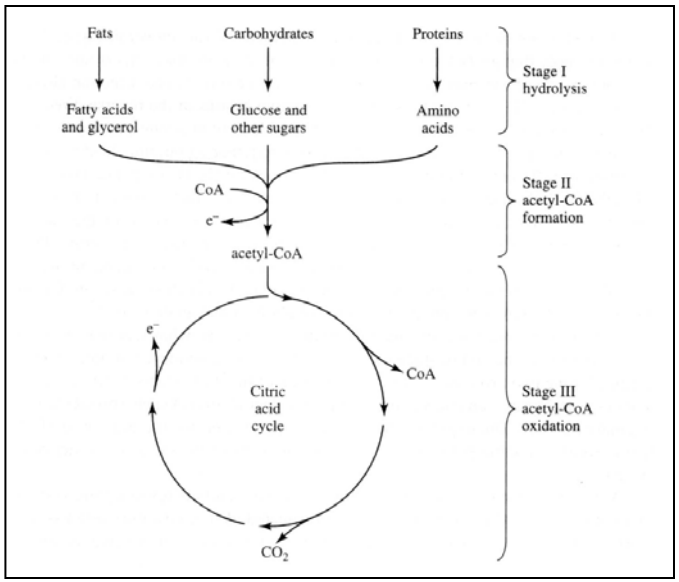
Energy coupling

Metabolism

Cells obtain energy via **oxidation of substrates** or through **photosynthesis**

chemotrophs phototrophs

chemoorganotrophs chemolithotrophs



→ Little if any energy

→ Some energy

→ Energy production

Anabolism

Catabolism

Depends on the oxidation and reduction of chemicals.

↓ e^- ↑ e^-

Oxidized material : electron donor (organic compounds, H_2, H_2S, NH_4^+)
Material being reduced : electron acceptor (O_2, NO_3^-, NO_2^-, Fe^{3+}, $SO_4^=$, CO_2)

Acetate and Oxygen (Aerobic Oxidation of Acetate)

$$Donor: \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- \qquad \Delta G^{0'} = -27.40kJ$$

$$Acceptor: \quad \frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O \qquad \Delta G^{0'} = -78.72kJ$$

$$Net: \quad \frac{1}{8}CH_3COO^- + \frac{1}{4}O_2 = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + \frac{1}{8}H_2O \qquad \Delta G^{0'} = -106.12kJ$$

Catabolism

Acetate and Carbon Dioxide (Methanogenesis of Acetate)

$$Donor: \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- \qquad \Delta G^{0'} = -27.40kJ$$

$$Acceptor: \quad \frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O \qquad \Delta G^{0'} = 23.53kJ$$

$$Net: \quad \frac{1}{8}CH_3COO^- + \frac{1}{8}H_2O = \frac{1}{8}CH_4 + \frac{1}{8}HCO_3^- \qquad \Delta G^{0'} = -3.87kJ$$

Glucose and Carbon Dioxide (Methanogenesis of Glucose)

$$Donor: \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^- \qquad \Delta G^{0'} = -41.35kJ$$

$$Acceptor: \quad \frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O \qquad \Delta G^{0'} = 23.53kJ$$

$$Net: \quad \frac{1}{24}C_6H_{12}O_6 = \frac{1}{8}CH_4 + \frac{1}{8}CO_2 \qquad \Delta G^{0'} = -17.82kJ$$

Catabolism of hydrocarbons, alcohols, aldehydes & ketones

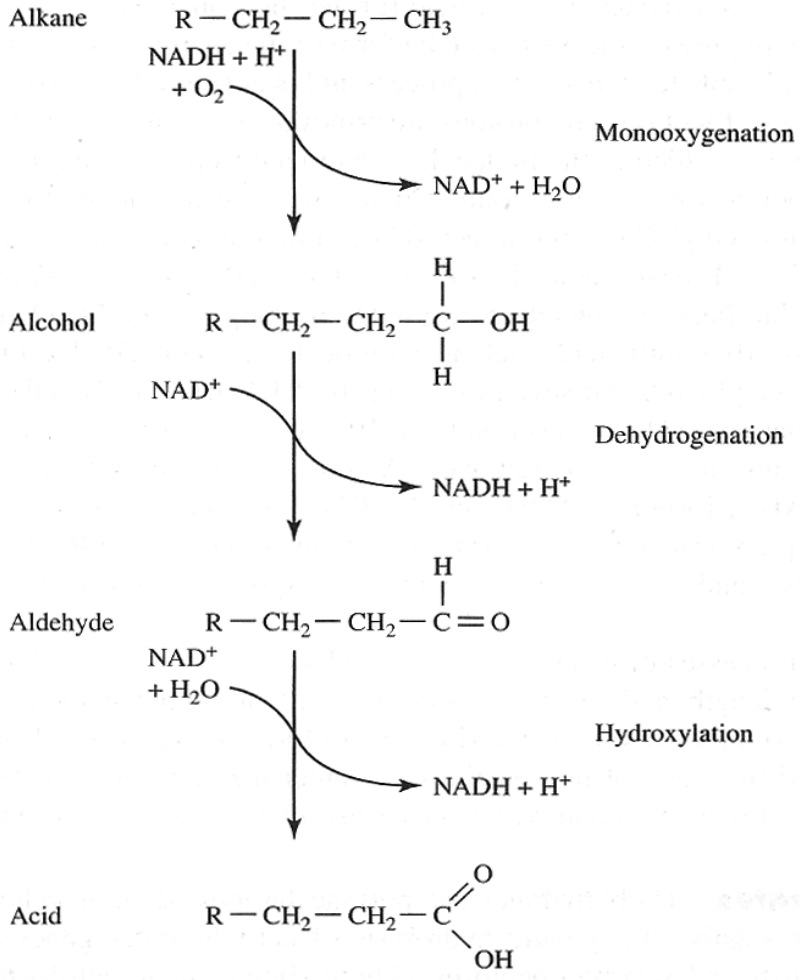
Hydrocarbons : either aliphatic or aromatic. Difficult to attack, chemically or biologically, because of the **C-H** or **C-C** bond

Oxygenation is an energy-costly reaction.

Organism yield per e-eq of hydrocarbon oxidized is lower than with other organic compounds.

When O_2 is not available organic compounds containing Oxygen or water can be used.

An **alkene** is converted to an **alcohol** by enzymatic addition of water in the double bond (C=C).



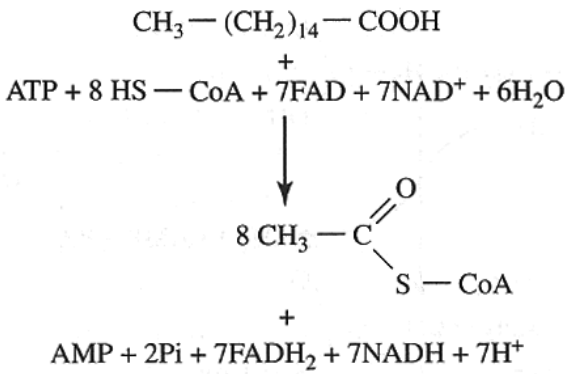
Catabolism of hydrocarbons, alcohols, aldehydes & ketones

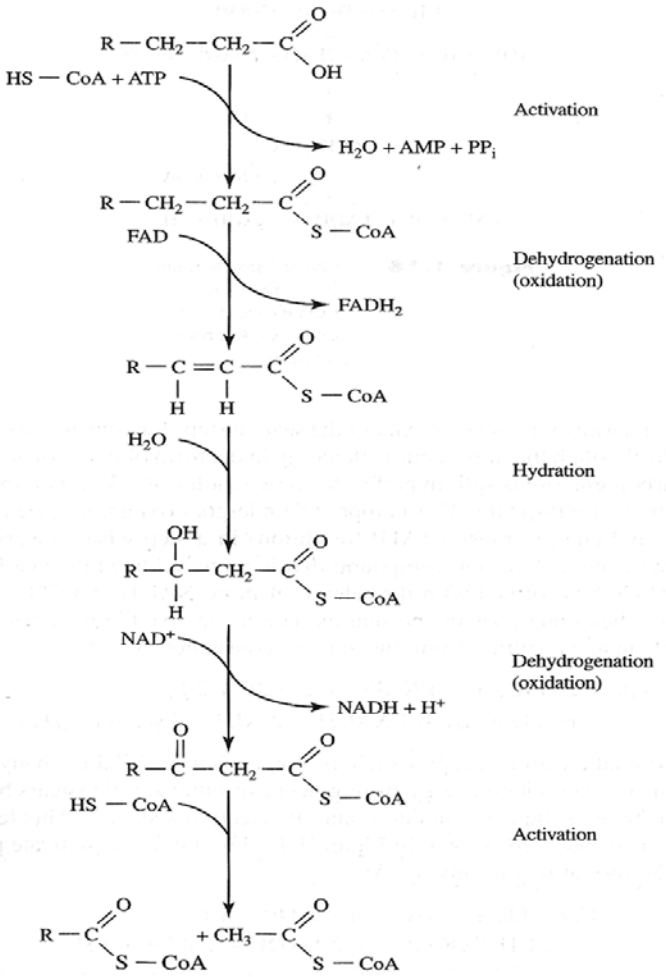
Fatty acids are oxidized by the process of **β-oxidation**.

Final products: **acetyl CoA, $FADH_2$, NADH**

acetyl CoA then enters the citric acid cycle.

Overall stoichiometry for oxidation of palmitic acid :





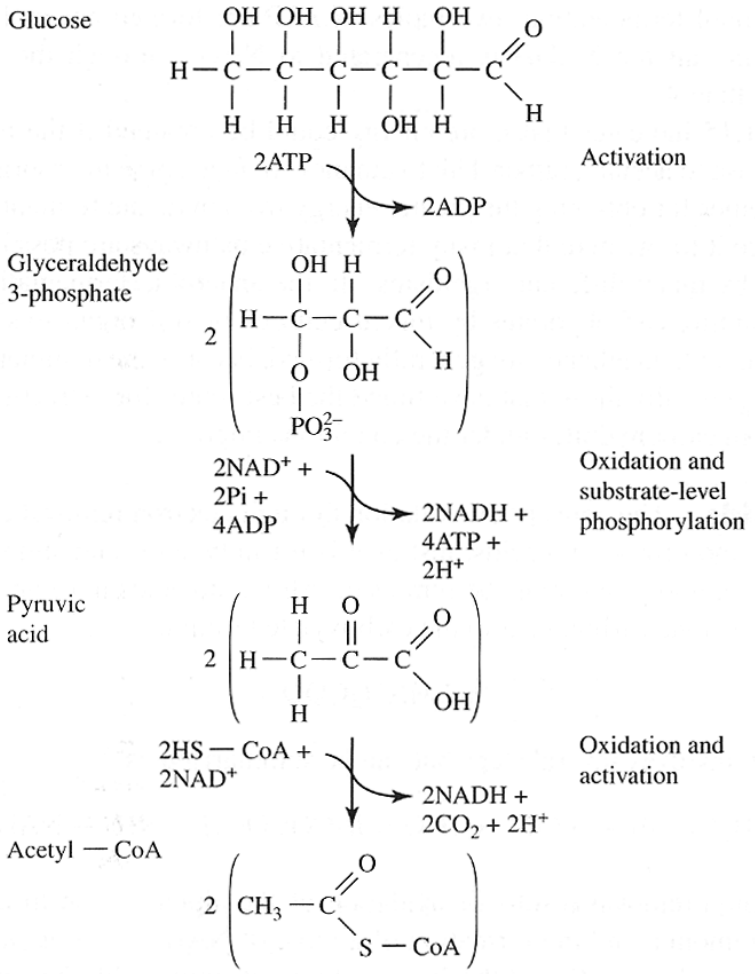
Catabolism of carbohydrates

Carbohydrates：polysaccharides such as cellulose, starch and complex sugars. Enzymatic hydrolysis produces 6-carbon hexoses or 5-carbon pentoses.

Carbohydrates are higher in energy content than other organic compounds. Even under fermentative conditions m/o$_s$ can obtain energy.

Catabolism of carbohydrates

EXAMPLE : conversion of glucose to acetyl coA



Overall stoichiometry :

$$C_6H_{12}O_6 + 2HSCoA + 4NAD^+ + 2ADP + 2P_i \rightarrow 2CH_3COSCoA + 4NADH + 2ATP + 2CO_2 + 4H^+$$

Fermentation & substrate-level phosphorylation :

$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P_i \rightarrow 2CH_3COCOO^- + 2NADH + 2ATP + 4H^+$$

Transferring the electrons of **NADH** back to pyruvate a lot of products may be formed : e.g. ethanol, propanol, propionate, butyrate, and H_2.

Catabolism of carbohydrates

EXAMPLE : **Ethanol production from glucose**:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

Acetaldehyde formation from pyruvate:

$$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$$

Ethanol formation from acetaldehyde and NADH:

$$CH_3CHO + NADH + H^+ \rightarrow CH_3CH_2OH + NAD^+$$

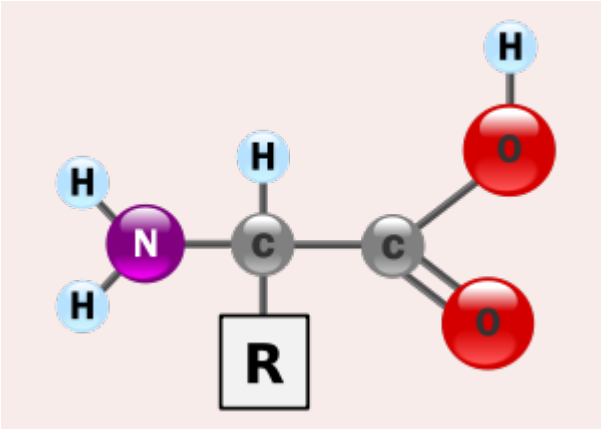
$$Net: C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2CH_3CH_2OH + 2CO_2 + 2ATP$$

More energy can be produced if acetic acid is produced instead of ethanol !!

Catabolism of amino acids

Amino acids are subjected to oxidation through electron removal and water addition.

General formula : $H_2NCHRCOOH$, where R is an organic **substituent**



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Deamination can be summarized as follows:

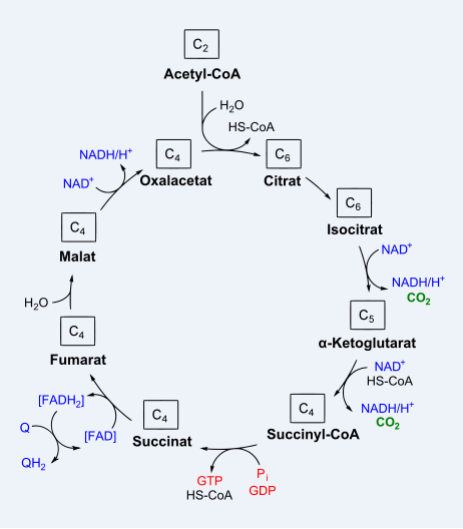
$$R-CHNH_2COOH + NAD^+ + H_2O = R-COCOOH + NH_3 + NADH + H^+$$

The organic acids produced may enter the citric acid cycle at various points.

Citric Acid Cycle

The most important features are these :

- The eight electrons in acetate are pairwise removed in four steps that generate three NADHs and one $FADH_2$.
- The two carbons in acetic acid are removed in two steps that produce $C0_2$.
- One step is a substrate-level phosphorylation that gives one GTP (guanosine triphosphate, an analog of ATP).
- Four steps add H_20, and one step removes H_20.
- In the last step, malate is oxidized to oxalacetate, which is then available to combine with acetyl CoA and begin the cycle again.



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Net result of all reactions :

$$CH_3COSCoA + 3NAD^+ + FAD + GDP + P_i + 3H_2O \rightarrow$$
$$2CO_2 + 3NADH + FADH_2 + GTP + 3H^+ + HSCoA$$

$$C_6H_{12}O_6 + 10NAD^+ + 2FAD + 2ADP + 2GDP + 4P_i + 6H_2O \rightarrow$$
$$6CO_2 + 10NADH + 2FADH_2 + 2GTP + 2ATP + 10H^+$$

Oxidative Phosphorylation

Oxidative phosphorylation is the means by which respiration generates **energy** for the cell. The amount of energy depends on the **final electron acceptor**.

O_2 : 3 moles ATP/ mole NADH **$SO_4^=$** : 1 moles ATP/ mole NADH

$ATP = ADP + Pi$ $\Delta G^0 = -32$ KJ/mole ATP

Pumping H^+ and OH^- ions to opposite directions of the cell membrane the cell creates a **proton motive force (PMF)** across the membrane. The PMF is a gradient in free energy that can be exploited to drive ATP formation from ADP and phosphate .

$$H^+(out) + ADP + Pi = H^+(in) + ATP$$

References

The images where their origin is not mentioned are derived from the book:

Environmental Biotechnology : Principles and Applications,

Bruce E. Rittmann and Perry L. McCarty,

McGraw-Hill Series in Water Resources and Environmental Engineering

Τέλος Ενότητας

Χρηματοδότηση

- Το παρόν εκπαιδευτικό υλικό έχει αναπτυχθεί στο πλαίσιο του εκπαιδευτικού έργου του διδάσκοντα.
- Το έργο «**Ανοικτά Ακαδημαϊκά Μαθήματα στο Πανεπιστήμιο Αθηνών**» έχει χρηματοδοτήσει μόνο την αναδιαμόρφωση του εκπαιδευτικού υλικού.
- Το έργο υλοποιείται στο πλαίσιο του Επιχειρησιακού Προγράμματος «Εκπαίδευση και Δια Βίου Μάθηση» και συγχρηματοδοτείται από την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο) και από εθνικούς πόρους.

Σημειώματα

Σημείωμα Ιστορικού Εκδόσεων Έργου

Το παρόν έργο αποτελεί την έκδοση 1.0.0.

Σημείωμα Αναφοράς

Σημείωμα Αδειοδότησης