SRINIVASAN COLLEGE OF ARTS & SCIENCE

(Affiliated to Bharathidasan University, Trichy)

PERAMBALUR – 621 212.

DEPARTMENT OF MICROBIOLOGY

Course : B.Sc Year: **I** Semester: **II**

Course Material on:

MICROBIAL METABOLISM

Sub. Code : 16SCCMB2

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MICROBIAL METABOLISM

CLASS: I B.SC., MICROBIOLOGY

SUBJECT CODE: 16SCCMB2

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MICROBIAL METABOLISM

OBJECTIVE

To understand the growth, enzymology and physiological processes of microbes

Unit I

Nutrition and growth of microorganisms: Nutritional types of microorganisms, nutritional requirements. Factors influencing the growth of microorganisms – temperature, pH, Osmotic pressure, moisture, radiations and different chemicals, Physiology of growth – Significance of various phases of growth. Growth measurements – batch, continuous and synchronous.

Unit II

Bacterial enzymes – classification, properties, kinetics of enzyme action – Michaelis Menton equation for simple enzymes - coenzymes and cofactors, isozymes.

Unit III

Metabolism of carbohydrates : Anabolism – phototsynthesis – oxygenic – an oxygenic, synthesis of carbohydrate – catabolism of glucose – Embden Mayer – Hoff – Parnas pathway – Pentose pathway, Kreb's cycle (TCA) – electron transport system and ATP production.

Unit IV

Metabolism of protein – synthesis and degradation of amino acids – glycine tyrosine, cysteine, serine, glutamine, synthesis of peptides and proteins – urea cycle.

Unit V

Anaerobic Respiration – Nitrate, sulphate and Methane respiration – Fermentations – alcohol, mixed acid, lactic acid fermentation – Metabolism of lipids – biosynthesis of fatty acids and cholesterol – oxidation of fatty acids.

To obtain energy and construct new cellular components, organisms, must have a supply of raw materials or nutrients. **Nutrients** – are substances used in biosynthesis and energy production.

Nutrient Requirements:

Microbial cell composition shows that 95% of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.

Macronutrients or macro elements:

These are required by microorganisms in relatively large amounts. Carbon, oxygen, hydrogen nitrogen, sulfurs and phosphorous are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.

K^+ - is required for the activity by a number of enzymes, including those involved in protein synthesis.

Ca^{2+} - contributes to the heat resistance of bacterial endospores. 15% of spore contains dipicolinic acid and calcium.

Mg^{2+} - serves as a cofactor for many enzymes, complexes with ATP and stabilizes ribosomes and cell membranes.

Fe^{2+} and Fe^{2+} - part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

Micronutrients or Trace elements:

These are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

Zn^{2+} - is present at the active site of some enzymes but is also involved in the association of regulatory and catalytic subunits in *E.coli* aspartate carbomoyl transferase.

Mn^{2+} - aids many enzymes catalyzing the transfer of phosphate groups.

Mo^{2+} - required for nitrogen fixation.

Co^{2+} - is a component of Vitamin B12.

Besides macro and micro nutrients, some microorganisms may have particular requirements that reflect the special nature of their morphology or environment. Diatoms need silicic acid to construct their beautiful cell walls of silica. Bacteria growing in saline lakes and oceans

depend on the presence of high concentrations of sodium ion. Microorganisms require a balanced mixture of all the above nutrients for proper growth.

Requirements for carbon, hydrogen and oxygen:

Carbon is needed for the skeleton or backbone of all organic molecules and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms. One important carbon source that does not supply hydrogen or energy is CO_2 .

Autotrophs – can use CO_2 as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source. Some autotrophs oxidize inorganic molecules and derive energy from electron transfer.

Heterotrophs – are organisms that use reduced pre-formed organic molecules as carbon sources. Ex. Glycolytic pathway produces carbon skeleton for use in biosynthesis and also releases energy as ATP and NADH. Actinomycetes will degrade amyl alcohol, paraffin and even rubber. *Burkholderia cepacia* can use over 100 different carbon compounds.

Some microorganisms can metabolize even relatively indigestible human-made substances such as pesticides. Indigestible molecules can be oxidized and degraded in the presence of a growth promoting nutrient that is metabolized at the same time, a process called Co-metabolism. The products of this breakdown can then be used as nutrients by other microorganisms.

Nutritional types of microorganisms:

In addition to Carbon, hydrogen and oxygen all organisms require sources of energy and electrons for growth.

Carbon sources:

Autotrophs - CO_2 sole or principal biosynthetic carbon source

Heterotrophs – reduced, preformed organic molecules from other organisms.

Energy sources:

Phototrophs – use light as their energy source.

Chemotrophs – obtain energy from the oxidation of chemical compounds (either organic or in organic)

Electron sources:

Lithotrophs – use reduced inorganic substances as their electron source.

Organotrophs – extract electrons from organic compounds.

Four major nutritional classes based on their primary sources of carbon, energy and electrons is known.

Phtotolithotrophic autotrophs or photoautotrophs or photolithoautotrophs:

Source of energy – light energy

Source of electrons – Inorganic hydrogen/ electron

Carbon source - CO_2

Example: Algae, purple and green sulfur bacteria and cyanobacteria.

Photoorganotrophic heterotrophy or photoorganoheterotrophy:

Source of energy – light energy

Source of electrons – organic hydrogen/ electron

Carbon source – organic carbon sources (CO_2 may also be used)

Example: Purple and green nonsulfur bacteria (common inhabitants of lakes and streams)

Chemolithotrophic autotrophs or chemolithoautotrophy:

Source of energy – Chemical energy source (inorganic)

Source of electrons – Inorganic hydrogen/ electron donor

Carbon source - CO_2

Example: Sulfur-oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron-oxidizing bacteria.

Chemoorganotrophic heterotrophs or chemoorganoheterotrophy:

Source of energy – Chemical energy source (organic)

Source of electrons – Inorganic hydrogen/ electron donor

Carbon source – organic carbon source

Example: Protozoan, fungi, most non-photosynthetic bacteria (including most pathogens)

The most common nutritional types are photolithoautotrophs and chemoorganoheterotrophs. Bacteria *Beggiatoa* rely on inorganic energy sources and organic (or sometimes CO_2) carbon sources. These microbes are sometimes called Mixotrophic because they combine chemolithoautotrophic and heterotrophic metabolic processes.

Requirements for nitrogen, phosphorous and sulfur:

Nitrogen is needed for the synthesis of amino acids, purines, pyramidines, some carbohydrates and lipids, enzyme cofactors and other substances. Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction. A variety of bacteria like many Cyanobacteria and *Rhizobiium* can reduce and assimilate atmospheric nitrogen using the nitrogenase systems. Phosphorous is present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components. All microorganisms use inorganic phosphate as their phosphorous source and incorporate it directly. *E.coli* can use both organic and inorganic phosphate. Organophosphates such as hexose 6- phosphate can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate which is then transported across the plasma membrane. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel. Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates biotin and thiamine. Most of them use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

FACTORS INFLUENCE THE GROWTH OF MICROORGANISMS

The growth of microorganisms is greatly affected by the chemical and physical nature of their surroundings. An understanding of these influences aids in the control of microbial growth and the study of the ecological distribution of microorganisms. Prokaryotes are present or grow anywhere life can exist. The environments in which some prokaryotes grow would kill most other organisms. For example *Bacillus infernus* is able to live over 1.5 miles below the earth's surface without O_2 and 60^oC temperature. These microorganisms which can thrive and grow in such harsh conditions are often called **extremophiles.**

The major physical factors which affect microbial growth are solutes and water activity, pH, temperature, oxygen level, pressure and radiation.

Solutes and Water activity: Changes in osmotic concentration of the surroundings can affect microbial growth as a selectively permeable plasma membrane separates the microorganisms from their surroundings. Microorganisms need to keep the osmotic concentration of their cytoplasm somewhat above that of the habitat by the use of compatible solutes, so that the plasma membrane is always pressed firmly against their cell wall. In a hypertonic environment, the prokaryotes increase their internal osmotic concentration through the synthesis or uptake of choline, proline, glutamic acid and other amino acids. A few prokaryotes like *Halobacterium salinarium* raise their osmotic concentration with potassium ions. The enzymes of these bacteria are altered for the requirement of high salt concentrations for normal activity. Halophiles grow optimally in the presence of NaCl or other salts at a concentration above about 0.2M. These have extensively modified the structure of their proteins and membranes rather than simply increasing the intracellular concentrations of solutes. They require higher potassium levels for stability and activity. The plasma membrane of halophiles is also stabilized by high concentration of sodium ions.

Water activity (a_w) is the amount of water available to microorganisms and this can be reduced by interaction with solute molecules (osmotic effect). Water activity is inversely related to osmotic pressure; if a solution has high osmotic pressure, it's a w is low. Microorganisms differ greatly in their ability to adapt to habitats with low water activity. In a low a w habitat, the microorganisms must expend extra effort to grow as it should maintain a high solute concentration to retain water. Such microorganisms are osmotolerant or can grow over wide range of water activity or osmotic concentration. Most of the microorganisms grow at a w =0.98 or higher.

pH: It refers to the acidity or alkalinity of a solution. It is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithm of the hydrogen ion concentration.

$pH = -\log [H^+] = \log (1/H^+)$

The pH scale ranges from 1.0 to 14.0 and most microorganisms grow vary widely from pH 0 to 2.0 at the acid end to alkaline lakes and soil that may have pH values between 9.0 and 10. The pH can affect the growth of microorganisms and each species has a definite pH growth range and pH growth optimum. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles between 5.5 and 8.0 and alkalophiles prefer pH range of 8.5 to 11.5. Most bacteria and protozoans are neutrophiles, fungi prefer acid surroundings about pH 4 to 6; algae also seem to favour slight acidity. *Cyanidium caldarium* (algae) and archaeon *Sulfolobus acidocaldarium* are inhabitants of acidic hot springs; both grow well around pH 1 to 3 and at high temperature. Drastic changes/variations in cytoplasmic pH can harm microorganisms by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins. Prokaryotes die if the internal pH drops much below 5.0 to 5.5. External pH alterations also might alter the ionization of nutrient molecules and thus reduce their availability to the organism. The microorganism needs to maintain a neutral cytoplasmic pH and for this the plasma membrane may be relatively impermeable to protons. Neutrophiles appear to exchange potassium for protons using an antiport transport system. Extreme alkalophiles maintain their internal pH closer to neutrality by exchanging internal sodium ions for external protons. The antiport systems probably correct small variations in pH. In case of too much acidity (below 5.5 to 6.0) *S. typhimurium* and *E.coli* synthesize an array of new proteins as part of what has been called as their acidic tolerance response. If the external pH decreases to 4.5 or lower, chaperones such as acid shock proteins and heat shock proteins are synthesized. Microorganisms can change the pH of their own habitat by producing acidic or basic metabolic waste products. In order to maintain the pH, buffers are often included in the media to prevent growth inhibition. Phosphate is commonly used buffer and a good example of buffering agent. Peptides and amino acids in complex media also have a strong buffering effect.

Temperature: Temperature profoundly affects microorganisms as the most important factor influencing the effect is temperature sensitivity of enzyme-catalyzed reactions. Beyond a certain point of higher temperature, slow growth takes place and damages the microorganisms by denaturing enzymes, transport carriers and other proteins. The plasma

membrane also is disrupted as lipid bilayer simply melts and the damage is such an extent that it cannot be repaired. At very low temperature, membranes solidify and enzymes don't work rapidly. In summary, when organisms are above their optimum temperature, both the function and cell structure is affected at low temperature, function is affected. The cardinal temperatures vary greatly between microorganisms. Optimum usually range from 0°C to as high as 75°C, where as microbial growth occurs at temperature extending from -20°C to over 120°C. Archaen *Geogemma barossii* grows anaerobically at 121°C. The major microbial groups differ from one another regarding their maximum growth temperature. Upper limit for protozoans is around 50°C, some algae and fungi can grow at temperatures as high as 55°C to 60°C.

Microorganisms are classified into five classes based on their temperature ranges for growth.

1. **Psychrophiles:** Microorganisms grow well at 0°C and the optimum growth temperature of 15°C or lower and maximum at around 20°C. These microorganisms are isolated from Arctic and Antarctic habitats. They have adapted to their environment in several ways. Their enzymes, transport systems and protein synthetic mechanisms function well at low temperatures. The cell membranes have high levels of unsaturated fatty acids and remain semifluid when cold. At higher than 20°C, the psychrophiles begin to leak cellular constituents because of cell membrane disruption. Microorganisms such as *Pseudomonas, Vibrio, Alcaligenes, Bacillus, Arthrobacter, Moritella, Photobacterium* belong to this group. The psychrophilic *Chlamydomonasnivalis* turns a snowfield or glacier pink with its bright red spores.

2. **Psychrotrophs or Facultative Psychrophiles:** In this group many **s** pecies can grow at0 o C to 7°C, optimum between 20°C and 30°C. The spoilage of refrigerated foods is mainly caused by microorganisms belonging to this group.

3. **Mesophiles:** Growth optimum around 20°C to 40°C, minimum at 15°C to 20°C and maximum at 45°C or lower. Most of the organisms fall under or within this category including human pathogens.

4. **Thermophiles:** The microorganisms in this group can grow at temperature of 55°C or higher, minimum is usually around 45°C and growth optima at around 55°C to 65°C. Mostly prokaryotes and a few algae and fungi belong to this group. The habitats in which they grow include, composts, self-heating haystacks, hot water lines and hot springs. Microorganisms have more heat-stable enzymes and proteins synthesis systems, which function at high temperature. Heat stable proteins have high organized, hydrophobic interiors, more hydrogen bonds and other non-covalent bonds strengthen the structure. Amino acids like proline make the polypeptide chain less flexible and chaperones also aid in folding of proteins to stabilize them. DNA also is stabilized by specific histone like proteins. The membrane lipids are also stable and tend to be more saturated, more branched and of higher molecular weight. Archaeal thermophiles have membrane lipids with ether linkages, which protect the lipids from hydrolysis at high temperatures.

5. **Hyperthermophiles:** Few microorganisms can grow at 96°C or above and have maximum at 100°C; and growth optima between 80°C and about 113°C. *Pyrococcus* and *Pyrpdictiumoccultum* are examples of marine hyperthermophiles found in hot floors of the sea floor.

Oxygen Concentration: An aerobe is an organism able to grow in the presence of atmospheric O_2 and the ones that grow in its absence is an **anaerobe**. Organisms which completely are dependent on atmospheric O_2 for growth are **obligate aerobes**, and it serves as the terminal electron acceptor for the electron transport chain in aerobic respiration and employs it in the synthesis of sterols and unsaturated fatty acids. Organisms which do not require O_2 for growth but do grow better in its presence are called **facultative anaerobes** . **Aerotelerant anaerobes** such as *Enterococcus faecalis* simply ignore O_2 and grow equally well whether it is present or not. **Obligate anaerobes** like *Bacteroides, Fusobacterium, Clostridium pasteurianum, Methanococcus, Neocallimastix*, do not tolerate O_2 at all and die in its presence. Aerotelerant and obligate anaerobes cannot generate energy through respiration and must employ fermentation or anaerobic respiration pathways for the purpose. **Microaerophiles** are those organisms that are damaged by the normal atmospheric levels of O_2 (20%) and require O_2 levels between the range of 2% to 16% for growth. The nature of bacterial O_2 responses can be readily determined by growing the bacteria in culture tubes filled with a solid culture medium or a special medium like thioglycollate broth, which contains a reducing agent to lower O_2 levels. Aerobic microorganisms are cultured, either the culture vessel is shaken to aerate the medium or sterile air is pumped. Anaerobic microorganisms require special anaerobic media containing reducing agents such as thioglycollate or cysteine may be used. Removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas is also preferred. Co_2 and nitrogen is added to the chamber since many anaerobes require a small amount of Co_2 for best growth. The technique in which gas pak jar is used can be used.

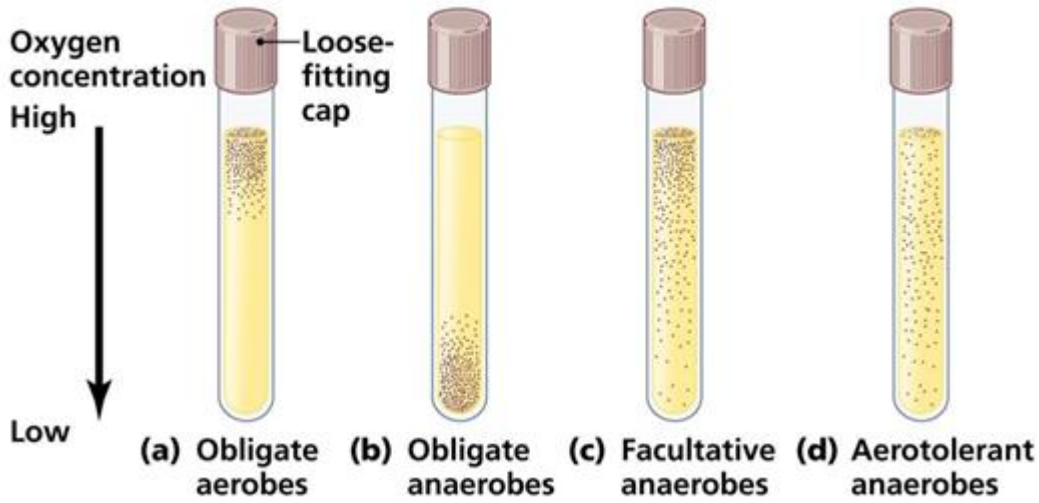


Fig. Oxygen requirements in bacteria

Pressure:

Most organisms on land or on the surface of water is always subjected to a pressure of 1 atm. The hydrostatic pressure can reach 600 to 1100 atm in the deep sea. Despite these extremes, bacteria survive and adapt. Many are barotolerant. Some bacteria in the gut of deep sea invertebrates such as amphipods and holothurians are truly barophilic and grow more rapidly at high pressures (Ex. *Photobacterium, shewanella, Colwellia*).

Osmotic Effects on Growth

1. Microbes require water to grow – their cells are 80 – 90% water
2. Water availability depends not only on amount of water present in any environment but also the concentration of solutes present (e.g., salts, sugars,…).
3. Water activity (a_w) - amount of water that is free to react = availability of water in a substance
4. a_w = a ratio of the vapour pressure of the air in equilibrium with a substance or solution to the vapour pressure of pure water (1/100 the relative humidity of a solution)
5. a_w ranges between 0 and 1
6. Most bacteria require an a_w of 0.9 for active metabolism
7. Most organisms are adversely affected by very low water activity (They suffer from plasmolysis)

In nature osmotic effects are of interest mainly in habitats with high salt concentration

Radiation:

Electromagnetic radiation of various types bombards our world. As the wavelength of electromagnetic radiation decreases, the energy of the radiation increases – gamma rays and X rays are much more energetic than visible light or infrared waves (Fig.). Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet radiation, infrared rays and radio waves. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun as visible light. Many forms of electromagnetic radiation are very harmful to microorganisms. Ionizing radiation, radiation of very short wavelength or high energy can cause atoms to lose electrons or ionize. The two major forms of ionizing radiation, X rays which are artificially produced and gamma rays which are emitted during radioisotope decay. Low levels of ionizing radiation will produce mutations, higher levels are directly lethal. Some prokaryotes like *Deinococcus radiodurans* and bacterial endospores are resistant and can cause a variety of changes in cells like; it breaks hydrogen bonds, oxidises double bonds, destroys ring structures and polymerizes some molecules. Oxygen enhances these destructive e effects, probably through the generation of hydroxyl radicals (OH.). Destruction of DNA is the most important cause of death of microorganisms. Ultraviolet radiation kills all kinds of microorganisms due to its short wavelength (approximately 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. Formation of thymine dimmers in DNA is the primary mechanism of UV damage; these dimmers inhibit DNA replication and function. This damage is repaired by photo reactivation, where blue light is used by a photo reactivating enzyme (photolyase) to split the thymine dimmers. Dark reactivation, where a short sequence containing the thymine dimmers can also be excised and replaced in the

absence of light. Damage can also be repaired by the recA protein in recombination repair and SOS repair.

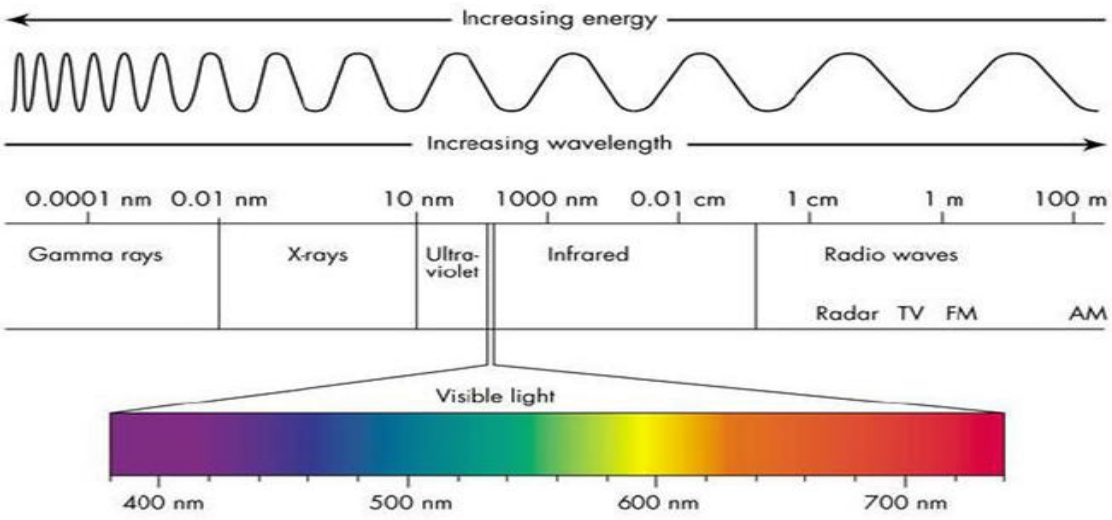


Fig. Electromagnetic spectrum

Visible light – immensely beneficial becuas eit is the source of energy for photosynthesis. Visible light when present in sufficieint intensity can damage or kill microbial cells. Pigments called photosensitizers and O_2 are required. All microorganisms possess pigments like chlorophyll, bacteriochlorophull, cytochromes and flavins which absorb light energy, become exited or activated and act as photosensitizers. The excited photosensitizer (P) transfers its energy to O_2 generating singlet oxygen ($'O_2$).

$$P\ (light) \rightarrow P(activated)$$

$$P(activated) + O_2 \rightarrow P + {}'O_2$$

Singlet oxygen is very reactive, powerful oxidizing agent that will quickly destroy a cell. Many microorganisms that are airborne or live on exposed surface use carotenoid pigments for protection against photooxidation. Carotenoids effectively quench singlet oxygen that is absorbing energy from singlet oxygen and convert it back into the unexcited ground state.

Microbial Growth Phases

Bacterial Growth Curve :

When bacteria are inoculated into a liquid growth medium, we can plot of the number of cells in the population over time.

Four phases of Bacterial Growth:

1.Lag Phase:

1. Period of adjustment to new conditions.
2. Little or no cell division occurs, population size doesn't increase.

3. Phase of intense metabolic activity, in which individual organisms grow in size.
4. May last from one hour to several days.

2. Log Phase:

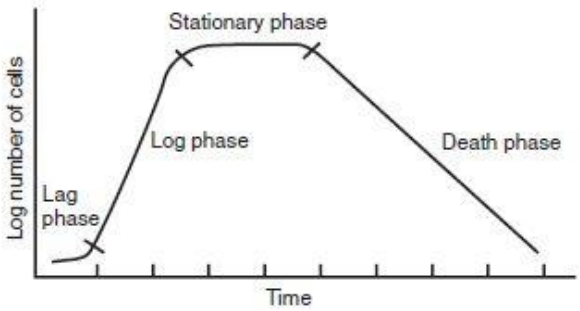
1. Cells begin to divide and generation time reaches a constant minimum. 2. Period of most rapid growth. Number of cells produced > Number of cells dying
3. Cells are at highest metabolic activity.
4. Cells are most susceptibleto adverse environmental factors at this stage. (Radiation and Antibiotics)

3. Stationary Phase:

1. Population size begins to stabilize. Number of cells produced = Number of cells dying.
2. Overall cell number does not increase.
3. Cell division begins to slow down.
4. Factors that slow down microbial growth: (Accumulation of toxic waste materials, Acidic pH of media, Limited nutrients and Insufficient oxygen supply)

. 4. Death or Decline Phase:

1. Population size begins to decrease. Number of cells dying > Number of cells produced.
2. Cell number decreases at a logarithmic rate.
3. Cells lose their ability to divide.
4. A few cells may remain alive for a long period of time.



Measuring Microbial Growth

Measurement of Growth

- Enumeration of microbial populations or measuring mass

i) Measurement of Cell Numbers

a) Direct Counting (counts all cells - viable and dead)

Direct microscopic counts with counting chambers (Fig 6.20)

- Use a chamber (e.g., Petroff-Hausser counting chamber) of defined volumes. Count cells the aid of a microscope
- can also use samples dried onto slides

Advantages

- rapid
- counts all cells in a sample (can often count individual cells in clumps)
- can acquire cell morphology information with these methods

Disadvantages

- can't determine which cells are viable unless they are treated in a special manner (e.g.,fluorescent live/dead cell stains).
- small cells are difficult to see
- affected by debris in samples
- not suitable for cell suspensions of low density (< 106/mL); precision difficult to achieve
- motile cells are difficult to count
- phase contrast microscopy required if sample not stained
- may require expensive pieces of equipment
- unable to perform further studies on the observed microbes without further cultivation

Filtration

- known volume of a suspension filtered onto a black polycarbonate filter membrane.
- cells are stained with fluorescent dyes and counted under the microscope

Coulter Counter

- automated method of counting cell.
- as cell pass through a aperture they disturb an electric field
- perturbations are transformed into number and size data.
- Most useful for larger cells

Fluorescence Activated Cell Sorter (FACS)

b) Viable Counts (counts viable cells that can be cultured)

Viable Plate Count

- counts viable cultivable bacteria
- Viable count methods assume that each viable cell can grow and divide to yield one colony
- Serial dilutions of cultures are prepared and these suspensions of bacteria are plated onto agar medium
- use spread plate or pour plate technique
- Following incubation - count number of colonies in order to determine the number of colony forming units (CFUs) per unit volume.
- limit counting to plates with between 30 and 300 colonies
- plates containing less than 30 colonies are not acceptable for statistical reasons
- plates containing greater than 300 (TNTC) - plates are crowded and it becomes hard to distinguish and count colonies.
- Problems with culturability of particular microbes on the medium - may be selective!!!!

Spread Plate

- suspension of microbes is spread over the surface of agar medium.
- spreading separates cells that grow and give rise to isolated colonies
- assumes each colony arises from a single cell or clump of cells (CFU).
- suspension of cells must be dilute enough otherwise the plate will be overgrown - too many cell get confluent growth or a lawn of cells with no discrete colonies.
- Usually spreading 0.1 mL of less on the plate

Pour Plate

- suspensions of cells (0.1 to 1.0 mL) are added to molten agar (42 to 45°C)
- Note - agar begins solidifies at approx. 42°C.
- molten agar is poured into a petri dish, allowed to solidify and incubated; the hot agar may kill or injure sensitive cells

Advantages of viable plate counts

- Counts only viable cells – widely used in food, dairy, medical industries and research
- Very sensitive – detect presence of very few cells
- Use of selective and/or differential media can restrict counts to a particular cell type
- the techniques require inexpensive materials
- once counts are completed you have viable cultures to use in subsequent experiments

Disadvantages of plate counts

- these methods are selective and count only viable cells or cells that can be grown with the culture techniques used (i.e., they underestimate actual cell number)
- they do not distinguish between an individual cell and a cluster of cells and therefore underestimate cell numbers

- takes time for data acquisition (i.e., Cells must grow for >12 h to be counted with the viable count methods
- size of colonies vary and it is easy to miss small colonies
- subject to large errors if not done carefully – require adequate replication

Most Probable Number (MPN)

- another technique for counting viable CFU
- dilute to extinction - such that not all aliquots transferred to tubes of growth medium will contain a cell
- following incubation one checks for growth and compares results to a table of statistical probability for obtaining the observed results.

Membrane filtration

- Aquatic samples are filtered through a membrane – trapping cells on the membrane
- The membrane is placed on an agar medium and incubated until each cell forms a colony
- Useful for analyzing water samples especially when the populations are low

c. Indirect estimation of Bacterial Numbers

Microbial Dry Weight

- Cells growing in liquid medium are collected by centrifugation or filtration, washed, dried in a vacuum oven and weighed
- Time consuming, not very sensitive but good for filamentous fungi

Turbidity (Spectophotometry)

- rapid and sensitive method for obtaining estimate of culture density
- The more cells that are present $\rightarrow$ the more light that is scattered by a suspension
- can measure transmittance of light and determine the optical density (OD) of a suspension using a spectrophotometer
- growth results in increased turbidity and OD $\rightarrow$ proportional to cell number for unicellular organisms

Can generate a standard curve to relate OD to CFU's/unit volume or some other measure of growth (e.g., dry weight)

Metabolic Activity

- Measures a metabolic product and assumes there is a direct relationship between the amount of the metabolic product and the cell number.
- Measurement of CO_2 evolution

Chemically defined media : Is one in which all the ingredients are known; and was prepared in the laboratory by adding a certain number of grams of each of the components (Carbohydrates, amino acids, salts etc). Particularly photolithoautotrophs such as Cyanobacteria and eukaryotic algae can be grown on relatively simple media containing CO_2 as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate and a variety of minerals. Chemoorganoheterotrophs can be grown in a defined media with glucose as a carbon source and an ammonium salt as a nitrogen source.

Complex media : contains undefined ingredients and the exact contents are not known. Complex medium may be sufficiently rich and complete to meet the nutritional requirements of many different microorganisms. They contain undefined components like peptones, meat extract and yeast extract. Peptones – are proteins hydrolysates prepared by partial proteolytic digestion of meat, casein, soyameal, gelatin and other protein sources (Carbon, energy and nitrogen). Beef extract – aqueous extracts from lean beef and contain amino acids, peptides, nucleotides, organic acids and minerals and vitamins.Yeast extract – from brewer's yeast and contain an excellent source of B vitamins, nitrogen and carbon compounds. Three commonly used complex media are

1. Nutrient broth – Peptone (5.0g/L), Beef Extract (3.0g/L)

2. Tryptic soya broth – Tryptone (enzyme digest of casein), Peptone (enzyme digest of soybean meal), glucose, NaCl and K_2HPO_4

3. MacConkey Agar – Pancreatic digest of gelatin, casein, peptic digest of animal tissue, bile salts mixture, NaCl, neutral red, crystal red and agar.

These media are routinely used for cultivation of bacteria in the laboratory and particularly useful for cultivation of bacteria whose growth requirements have not been defined.

Culture media can also be categorized as liquid or solid. Liquid media (also called broths) are contained in tubes, flasks and fermenters. Solid media are prepared by adding agar to liquid medium and then pouring the media into tubes or Petridishes where the media will solidify. Agar is a complex polysaccharide that is obtained from red marine algae. Other solidifying agents are gelatin, silica. A 1% or 2% agar can be used to solidify, but 1.5% is the most commonly used.

Enriched Medium : Is a broth or solid medium containing a rich supply of special nutrients that promotes the growth of fastidious organisms (that have complex nutrition and environmental requirements). It is usually prepared by adding extra nutrients to a medium called nutrient agar. Blood agar (nutrient agar + 5% sheep red blood cells): It is bright red in color and distinguishes between the hemolytic and non-hemolytic bacteria (*Streptococci* and other pathogens)

Chocolate agar (nutrient agar + powdered hemoglobin): It is brown in color and is considered more enriched than blood agar as hemoglobin is more readily accessible in chocolate agar. Pathogens like *Neisseria gonorrhoeae* and *Haemophillus influenza*, which will not grow on blood agar, can be cultured.

Types of Media:

Selective Media: Are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes.

Examples: MacConkey agar inhibits growth of gram positive bacteria and thus is selective for gram-negative bacteria. Phenylethyl alcohol (PEA) agar and Colistinalidixic acid (CAN) agar are selective for gram positive bacteria.

Manitol salt agar contains a concentration of 7.5% NaCl that is quite inhibitory to most human pathogens. Bile salts, a component of fecus, inhibit most gram positive bacteria while permitting many gram negative rods to grow. This media is used for selecting intestinal pathogens which contain bile salts. Dyes such as methylene blue and crystal violet also inhibit certain gram positive bacteria (Staphylococcus can produce acid from mannitol and turn the phenol red dye to bright yellow.

A medium containing acetate as a carbon source would be selective for organisms that grow on acetate. Sabaraud's dextrose agar is used to isolate fungi and cellulose for cellulose digesting bacteria.

Differential media : Makes it easier to distinguish colonies of the desired organism from other colonies growing on the same plate.

MacConkey agar is also a differential and selective medium to distinguish between lactose fermenting organisms (e.g, *E.coli*) and other non-fermenters (e.g, *Shigella* sp.). Lactose in the medium is fermented by *E.coli* producing acid which causes an indicator dye to change color to red and colonies that do not ferment lactose are white. Dyes can be used as differential agents because many of them are pH indicators that change color in response to the production of an acid or base. MacConkey agar contains neutral red, a dye that is yellow when neutral and pink or red when acidic.

Mannitol salt agar is used to screen for *Staphylococcus aureus*, and it turns the originally pink medium to yellow due to its ability to ferment mannitol. In a sense, blood agar is also differential because it is used to determine the hemolytic and non-hemolytic bacteria.

Blood agar is both differential and an enriched one. It distinguishes between hemolytic and non-hemolytic bacteria. Hemolytic bacteria (eg., many *Streptococci* and *Staphylococci*) produce clear zones around their colonies because of red blood cell destruction.

Isolation of pure cultures:

In natural habitats, microorganisms usually grow in complex, mixed populations containing several species. One needs a pure culture, a population of cells arising from a single cell, to characterize an individual species. Robert Koch introduced pure culture techniques.

Spread plate and streak plate:

Spread plate - Mixture of cells is spread out on an agar surface so that every cell grows into a completely separate colony, each colony representing a pure culture. A small volume of around 30 to 300 cells (mixed) is transferred to the center of the plate and spread evenly over the surface with a sterile bent-glass rod.

Streak plate – the microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. In both these techniques, successful isolation depends on spatial separation of single cells.

The pour plate : Extensively used with bacteria and fungi. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. The microbes are mixed with molten agar which has been cooled t 45° Cand then poured into pertidishes. All these techniques require petridishes (special culture dishes) after their inventor Julius Richard Petri (1887). They consist of two round halves, the top half overlapping the bottom. Each bacterium replicates to form a colony that is visible to the naked eye. A colony contains up to 109 copies of the original bacterium.

TYPES OF MICROBIAL CULTURE:

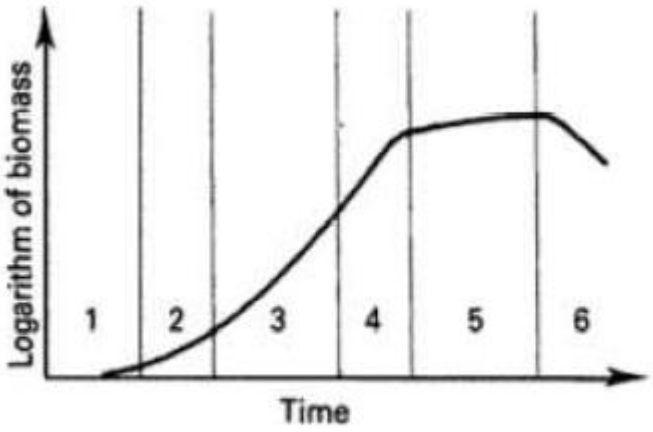
Microbial culture processes can be carried out in different ways. There are three models of fermentation used in industrial applications: batch, continuous and fed batch fermentation.

1. Batch Fermentation
2. Continuous Fermentation
3. Synchronous Growth

1.Batch Fermentation

A batch fermentation system is a closed system. At time t=0, the sterilized nutrient solution in the fermenter is inoculated with microorganisms and incubation is allowed to proceed at a suitable temperature and gaseous environment for a suitable period of time. In the course of the entire fermentation nothing is added, except oxygen (in case of aerobic microorganisms), an anti-foam agent, acid or base to control pH. The composition of the

medium, the biomass concentration and the metabolite concentration generally change constantly as a result of metabolism of the cells. After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, six typical phases of growth are observed.



Growth Characteristics In A Batch Culture Of A Microorganism,

Growth is a result of consumption of nutrients. The initial lag phase is a time of no apparent growth but actual biochemical analyses show metabolic turnover, indicating that cells are in the process of adapting to the environmental conditions and that new growth will eventually begin. There is then a transient acceleration phase as the inoculum begins to grow, which is quickly followed by an exponential phase. In the exponential phase, microbial growth proceeds at the maximum possible rate for that organism with nutrients in excess, ideal environmental parameters and growth inhibitors absent. However, in batch cultivation exponential growth is of limited duration and as nutrient conditions change, growth rate decreases, entering the deceleration phase, to be followed by the stationary phase, when overall growth can no longer be obtained owing to nutrient exhaustion. The final phase of the cycle is the death phase when growth rate has ceased. Most biotechnological batch processes are stopped before this stage because of decreasing metabolism and cell lysis. Typical microbial cultures in the laboratory (in a flask) are batch cultures.

Batch culture systems provide the following advantages and disadvantages:

Advantages

 Reduced risk of contamination or cell mutation as the growth period is short.
 Lower capital investment when compared to continuous processes for the same bioreactor volume.
 More flexibility with varying product/biological systems.
 Higher raw material conversion levels, resulting from a controlled growth period.

Disadvantages

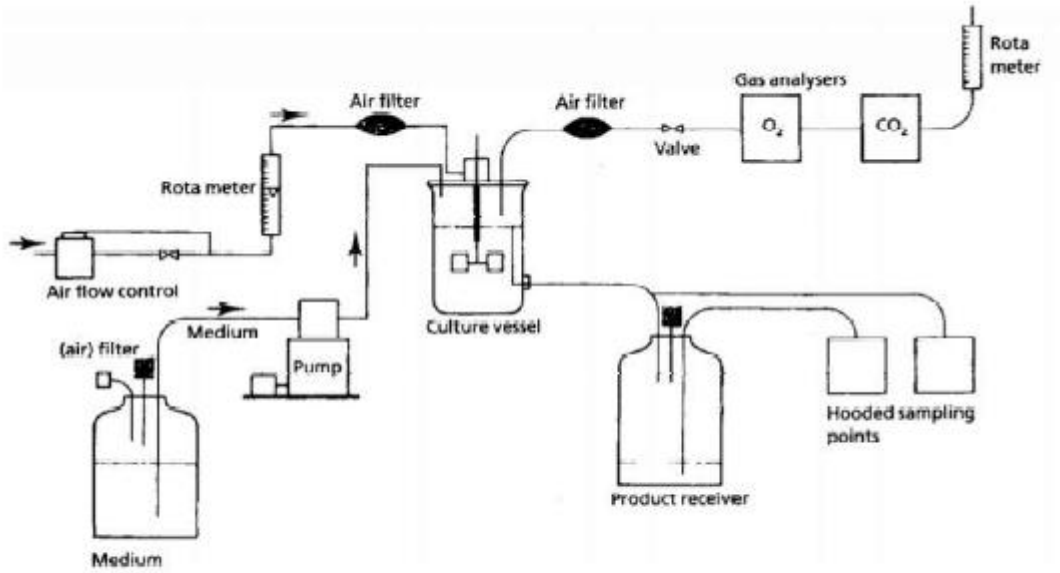
 Lower productivity levels due to time for filling, heating, sterilization, cooling, emptying and cleaning the reactor.

 Increased focus on instrumentation due to frequent sterilization.
 Greater expense incurred in preparing several subcultures for inoculation. Higher costs for labor and/or process control for this non-stationary procedure.

2.Continuous Fermentation

In continuous fermentation an open system is set up. Sterile nutrient solution is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system. In a homogeneously mixed bioreactor, we can have a chemostat or a turbidostat. In the chemostat, in the steady state adjusting the concentration of one substrate controls cell growth. In the turbidostat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.

In the chemostat, constant chemical environment is maintained, while in a turbidostat constant cell concentration is maintained. In a chemostat the growth chamber is connected to a reservoir of sterile medium. Once growth is initiated, fresh medium is continuously supplied from the reservoir. The volume of fluid in the growth chamber is maintained at a constant level by some sort of overflow drain. Fresh medium is allowed to enter the growth chamber at a rate that limits the growth of the bacteria. The rate of addition of fresh medium determines the rate of growth because the fresh medium always contains a limiting amount of an essential nutrient. Thus the chemostat relieves the insufficiency of nutrients, the accumulation of toxic substances and the accumulation of excess cells in the culture which are the parameters that initiate the stationary phase of the growth cycle. A simple laboratory fermenter operating on a continuous cultivation basis is shown in Figure.



A Simple Laboratory Fermenter Operating on a Continuous Cultivation Basis.

Advantages

 Continuous reactions offer increased opportunities for system investigation and analysis. As the variables remain unchanged, a benchmark can be determined for the process results, and then the effects of even minor changes to physical or chemical variables can be evaluated. By changing the growthlimiting nutrient, changes in cell composition and metabolic activity can be tracked. The constancy of the continuous process also provides a more accurate picture of kinetic constants, maintenance energy and true growth yields.

 Continuous culture provides a higher degree of control than a batch culture. Growth rates can be regulated and maintained for extended periods. By varying the dilution rate, biomass concentration can be controlled. Secondary metabolite production can be sustained simultaneously along with growth. In steady state continuous culture, mixed cultures can be maintained using chemostat cultures – unlike in a batch process where one organism usually outgrows another.

Disadvantages

 The control of the production of some non-growth related products is not easy. For this reason, the continuous process often requires feed-batch culturing and a continuous nutrient supply.

 Wall growth and cell aggregation can also cause wash-out or prevent optimum steady-state growth.

 The original product strain could be lost over time if a faster growing one overtakes it.

 The viscosity and heterogeneous nature of the mixture can also make it difficult to maintain filamentous organisms.

3.**Synchronous Growth**

Synchronous growth of a bacterial population is that during which all bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time. Synchronous growth helps studying particular stages or the cell division cycle and their interrelations.

In most of the bacterial cultures the stages of growth and cell division cycle are completely random and thus it becomes difficult to understand the properties during the course of division cycle using such cultures. To overcome this problem, the microbiologists have developed synchronous culture techniques to find synchronous growth of bacterial population.

Synchronous culture is that in which the growth is synchronous, i.e., all the bacterial cells of the population are physiologically identical and in the same stage of cell division cycle at a given time.

A synchronous culture can be obtained either by manipulating environmental conditions such as by repeatedly changing the temperature or by adding fresh nutrients to cultures as soon as they enter the stationary phase, or by physical separation of cells by centrifugation or filtration.

An excellent and most widely used method to obtain synchronous cultures is the Helmstetter-Cummings Technique (Refer Figure) in which an unsynchronized bacterial culture is filtered through cellulose nitrate membrane filter.

The loosely bound bacterial cells are washed from the filter, leaving some cells tightly associated with the filter. The filter is now inverted and fresh medium is allowed to flow through it. New bacterial cells, that are produced by cell division and are not lightly associated with the filter, are washed into the effluent. Hence, all cells in the effluent are newly formed and are, therefore at the same stage of growth and division cycle. The effluent thus represents a synchronous culture.

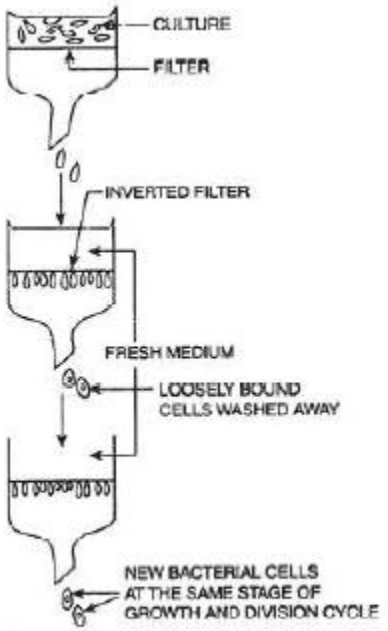


FIG. 19.4. Helmstetter-Cumming technique of obtaining synchronous cultures.

The Helmstetter-Cummings Technique

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Unit-II

Enzymes are the biological substance or biological macromolecules that are produced by a living organism which acts as a catalyst to bring about a specific biochemical reaction. These are like the chemical catalysts in a chemical reaction which helps to accelerate the biological/biochemical reactions inside as well as outside the cell. These are generally known as "Biocatalyst."

Enzyme classes and types of reactions:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

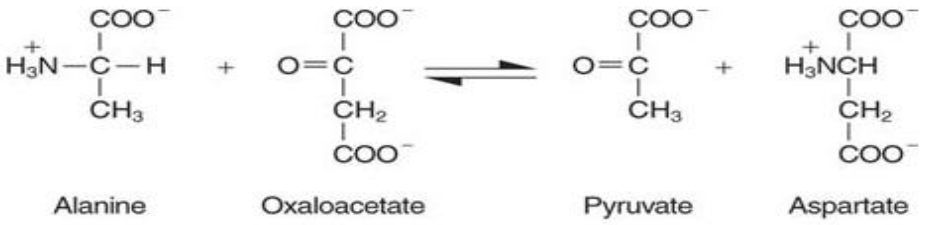
1.**Oxidoreductases:** Oxidation and reduction - Enzymes that carry out these reactions are called oxidoreductases. For example, alcohol dehydrogenase converts primary alcohols to aldehydes oxidoreductases.

For example, alcohol dehydrogenase converts primary alcohols to aldehydes.

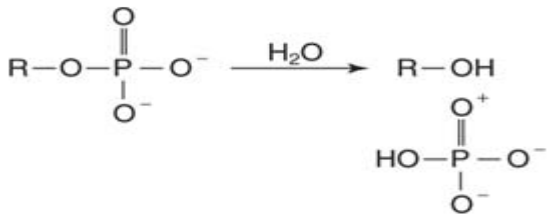
$$H_3CCH_2OH + NAD \rightleftharpoons H_3CCHO + NADH + H^+$$

In this reaction, ethanol is converted to acetaldehyde, and the *cofactor,* NAD, is converted to NADH. In other words, ethanol is oxidized, and NAD is reduced. (The charges don't balance, because NAD has some other charged groups.) Remember that in redox reactions, one substrate is oxidized and one is reduced.

2. Transferases : Group transfer reactions -These enzymes, called **transferases**, move functional groups from one molecule to another. For example, alanine aminotransferase shuffles the alpha-amino group between alanine and aspartate:



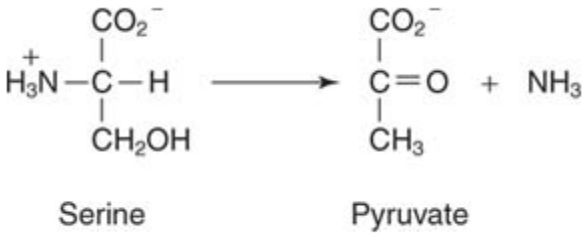
3. Hydrolysis. These enzymes, termed **hydrolases,** break single bonds by adding the elements of water. For example, phosphatases break the oxygen-phosphorus bond of phosphate esters:



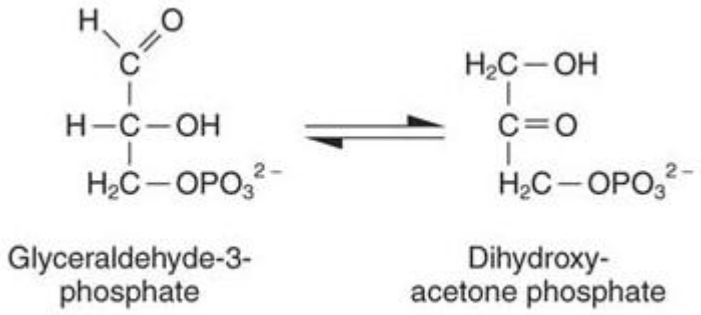
Other hydrolases function as digestive enzymes, for example, by breaking the peptide bonds in proteins.

4.Lyases : Formation or removal of a double bond with group transfer. The functional groups transferred by these **lyase** enzymes include amino groups, water, and ammonia. For example, decarboxylases remove CO_2 from alpha- or beta-keto acids:
Dehydratases remove water, as in fumarase (fumarate hydratase):

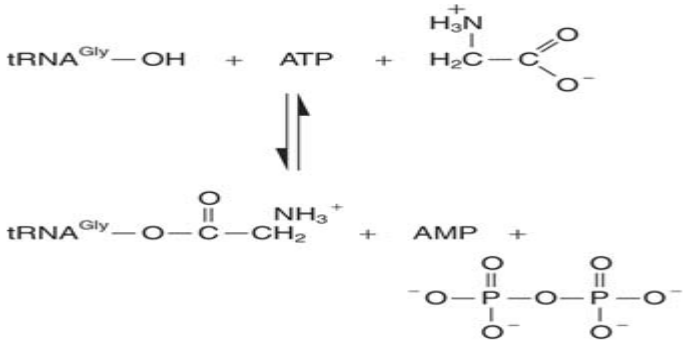
Deaminases remove ammonia, for example, in the removal of amino groups from amino acids:



5.Isomerases : Isomerization of functional groups. In many biochemical reactions, the position of a functional group is changed within a molecule, but the molecule itself contains the same number and kind of atoms that it did in the beginning. In other words, the substrate and product of the reaction are *isomers*. The **isomerases** (for example, triose phosphate isomerase), carry out these rearrangements.



6.Ligases : Single bond formation by eliminating the elements of water. Hydrolases break bonds by adding the elements of water; **ligases** carry out the converse reaction, removing the elements of water from two functional groups to form a single bond. Synthetases are a subclass of ligases that use the hydrolysis of ATP to drive this formation. For example, *aminoacyl-transfer RNA synthetases* join amino acids to their respective transfer RNAs in preparation for protein synthesis.



Cofactor: A non-protein chemical component required for proteins biological activity are called co-factor.

Apoenzyme: The protein part of an active enzyme is called apoenzyme.

Holoenzyme: the active enzyme composed of Apoenzyme and a co-factor is termed as holoenzyme.

Coenzyme: coenzyme is a non –protein compound or substance that is necessary for an enzyme to initiate the function of the enzyme.

Prosthetic group: A coenzyme or metal ion that is very tightly or even covalently bound to the protein component of the enzyme is called a prosthetic group.

Properties of Enzymes:

1. Simple enzymes are made up of a protein part alone and they are otherwise called **apoenzymes.** Complex enzymes are made up of a protein part and a small molecule of nonprotein. They are otherwise called as **Holoenzyme.**

2. If a protein and non protein part (cofactor) bind tightly and mediate a reaction, it called as prosthetic group.
3. Enzymes are highly specific in their catalytic action.
4. The enzymes contains an area called the active site that reacts with the substrate.
5. Enzymes are never consumed in the reaction. The recycle after the product is formed.
6. Specificity of enzymes**:** Enzymes are the most remarkable and highly specialized proteins, they have a high degree of specificity for their substrates, and they accelerate chemical reactions tremendously. In general, four types of behavior can be described:
    1. **Absolute specificity** - Catalyze only one reaction.
    2. **Group specificity** – catalyses a particular type of functional group, which can occur in a variety of substrate.
    3. **Linkage specificity** - Catalyses a particular type of chemical bond regardless of the rest of the molecular structure.
    4. **Stereochemical specificity** - the enzyme will act on a particular steric or optical isomer.

Enzymes are of two types, depending on the number of polypeptides. They are listed as follows:

(i) **Monomeric enzymes:** A monomeric enzyme is made of one polypeptide unit. Example: ribonuclease and trypsin.

(ii) **Oligomeric enzymes:** An oligomeric enzyme contains more than one polypeptide chain. Examples: lactate dehydrogenase, aspartate ranscarbamylase, and so on. Some enzymes are multi-enzyme complex. They have specific sites to catalyse different reactions in a sequence. The single specific site cannot complete its function. Only when they are in complex, they can run the reaction in series to ultimately obtain the product.

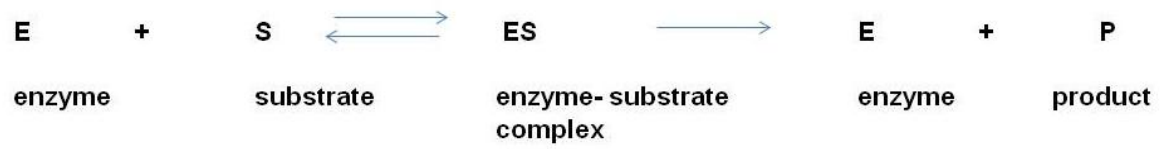
Kinetics of enzyme action

Enzymes differ widely in structure and specificity, but a general theory that accounts for their catalytic behavior is widely accepted.

The enzyme and its substrates interact only over a small region of the surface of the enzyme, called the active site.

– When the substrate binds to the active site via some combination of intermolecular forces, an enzyme-substrate (ES) complex is formed.

– Once the complex forms, the conversion of the substrate (S) to product (P) takes place:

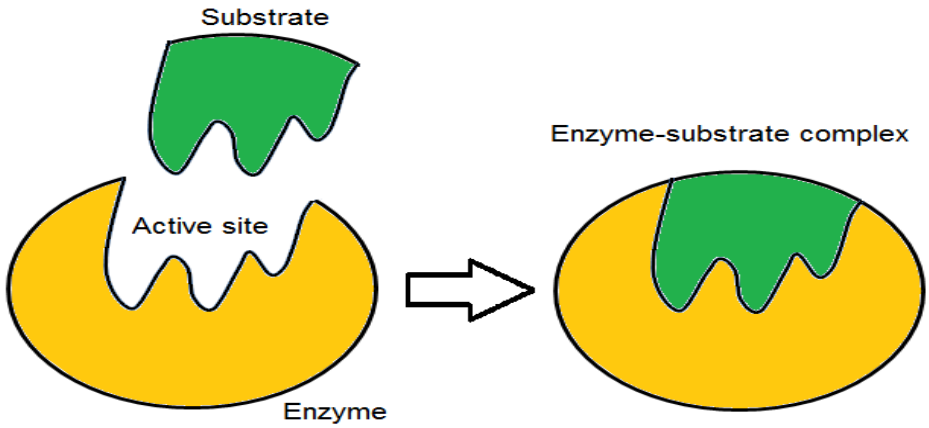


There are three different models that represent enzyme–substrate binding:

1. Lock-and-key model
2. Induced fit model
3. Substrate strain model

1. **Lock-and-Key Model:** The lock-and-key model was proposed by Emil Fischer in 1890. This model presumes that there is a perfect fit between the substrate and the active site—two molecules are complementary in shape. Lock-and-key is such a model that the active site of enzyme is a good fit for the substrate that does not require change of enzyme structure after the enzyme binds substrate. Thus, the active site has a pre-shaped template where only a specific substrate can bind. This model failed because it could not explain many aspects of enzymatic reactions and even could not explain the allosteric modulation.

   Lock-and-Key Model

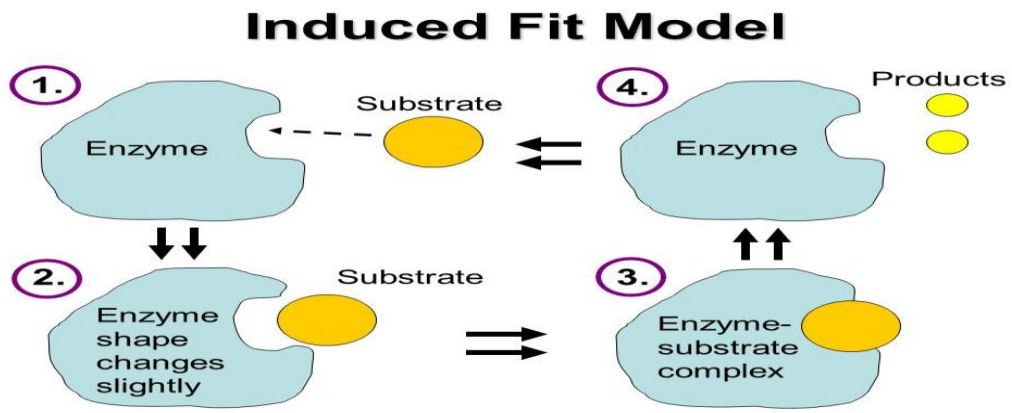


   The substrate and enzyme active site have complementary shapes.

2. **Induced fit model:** The induced fit model involves the changing the conformation of the active site to fit the substrate after binding. Also, in this model, it was stated that there are amino acids that aid the correct substrate to bind to the active site. This leads to lending shaping of the active site to the complementary shape.

   The induced fit model is such that the structure of the active site of enzyme can be easily changed after binding of enzyme and substrate. According to this model, the binding or active site is rigid as opposed to the lock-and-key model. The original or nascent active site has a different confirmation. However, on interaction with the substrate, the enzyme active site changes and substrate binds to enzyme very strongly. Thus, the substrate binding to enzyme induces a better enzyme substrate complex. Now it is known that amino acids of inactive sites are reoriented after interaction with enzymes and help in activating catalytic activity of the enzyme.

   The binding in the active site involves hydrogen bonding, hydrophobic interactions and temporary covalent bonds. The active site will then stabilize the transition state intermediate to decrease the activation energy. However, the intermediate is most likely unstable, allowing the enzyme to release the substrate and return to the unbound state.



Substrate strain model: According to this model, the substrate is strained due to induced change in the confirmation of the enzyme. When a substrate binds to a preformed active site, the enzyme induces a strain to the substrate. This strained substrate helps in the formation of a new product. Nowadays, combinations of both induced fit model and substrate strain models are used to explain the mechanism of enzyme action.

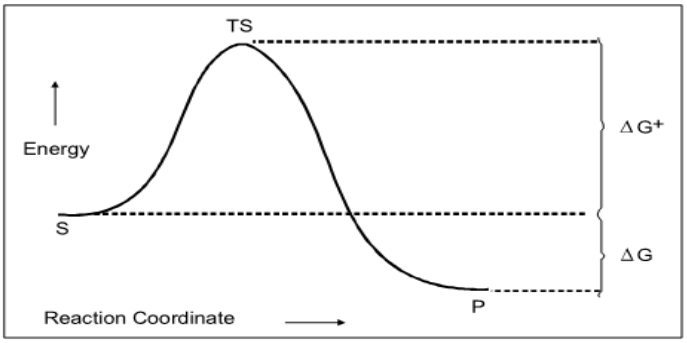
Simple Enzyme Kinetics:

Enzyme kinetics studies the reaction rates of enzyme-catalyzed reactions and how the rates are affected by changes in experimental conditions. Leonor Michaelis and Maud Menten were among the first scientist to experiment with enzyme kinetics in a "modern" way, controlling the pH of the solution etc.

$$E + S \longleftrightarrow ES \longleftrightarrow EP \longleftrightarrow E + P$$

Where E, S and P represent enzyme, substrate and product, respectively. ES and EP represent the complex of enzyme with substrate and product, respectively.

Process of a Reaction without an Enzyme



Symbols

S = free energy of substrate

P = free energy of the product

TS = transition state

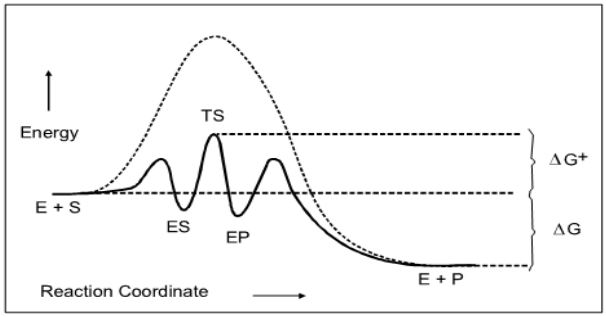
G^{*}= activation energy for the two reactions

G = overall standard free energy change in moving from S-P

The free energy of P is lower than that of S. Therefore, free energy change is negative for this reaction that favours P. But there exists an energy barrier between the S and P, which is required for the reaction to proceed in either direction. This energy barrier is called the transition state. For the reaction to proceed, the reactant must reach this energy barrier. The difference between the ground state and transition state is known as activation energy.

Therefore, activation energy and rate of reaction are inversely proportional to each other (i.e. if the activation energy is higher, the rate of reaction is slower and vice versa). To overcome this problem, a catalyst is used to enhance the rate of a chemical reaction or, in natural reactions, a catalyst is used by the system itself in the form of enzymes. Therefore, a catalyst enhances the rate of a reaction by lowering the activation energy.

Process of a Reaction with an Enzyme



Symbols

E = enzymes

ES = enzyme and substrate complex

EP = enzyme and product complex

The enzymes do not get used up in the reaction, and the equilibrium point remains unaffected. They only enhance the rate of a reaction. When there are more steps in the reaction, the overall rate is determined by the step with great activation energy, which is known as the rate-limiting step.

Michaelis-Menten Hypothesis

Leonor Michaelis was a German-born American biochemist and physician famous for his work in enzyme kinetics and Michaelis-Menten kinetics. Maud Leonora Menten was a Canadian medical scientist who made significant contributions to enzyme kinetics and histochemistry. They proposed a theory based on the following assumptions:

1. Only single substrate and single product are involved.
2. The reaction proceeds essentially to completion.
3. The concentration of substrate is much greater than that of the enzyme.
4. An enzyme–substrate complex is formed as an intermediate step.
5. The rate of decomposition of the substrate is proportional to the concentration of the enzyme–substrate complex. Consider the following reaction:

It is practically difficult to measure the quantity of ES or even S at any pointof the reaction. Therefore, Michaelis and Menten replaced these immeasurable quantities with practically measurable quantities.

The following symbols are used to derive Michaelis-Menten equation:

(E_t) = total concentration of enzyme

(S) = total concentration of substrate

(ES) = concentration of enzyme substrate complex

$(E_t) - (ES)$ = concentration of free enzyme

The rate of reaction is proportional to the concentration (V) of the enzyme–substrate complex.

$$V = k(ES) \quad ...(1)$$

When total enzymes (E_t) bound to the substrate, maximum rate of reaction occurs. At this point the maximum concentration of ES (V_m) is equal to the total concentration of enzyme:

$$V_m = k(E_t) \quad ...(2)$$

By dividing Equation (1) by Equation (2), Equation (3) is obtained:

$$V/V_m = (ES)/(E_t) \quad ...(3)$$

For the reversible reaction $E + S \longleftrightarrow ES$, the equilibrium constant for dissociation (k_m) of ES is as follows: $(E_t) - (ES) \times (S)$

$$K_m = \frac{(E_t) - (ES) \times (S)}{(ES)} \quad ...(4)$$

$$(ES) \times K_m = (E_t) \times (S) - (ES) \times (S)$$

$$(ES) \times K_m + (ES) \times (S) = (E_t) \times (S)$$

$$(ES) \times [K_m + (S)] = (E_t) \times (S)$$

$$(ES)/(E_t) = (S)/ K_m + (S) \quad ...(5)$$

Now, substitute the value of Equation (3) in Equation (5):

$$V/V_m = (S)/K_m + (S)$$

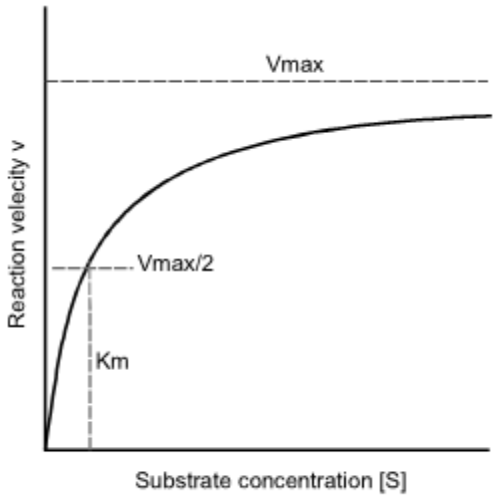
$$V = V_m [(S)/K_m + (S)] \quad ...(6)$$

$$K_m = (S) [V_m/V - 1] \quad ...(7)$$

Where K_m = Michaelis-Menten constant

Equation (6) is the Michaelis-Menten equation, which can be used to calculate K_m after experimentally determining the rate of reaction at various substrate concentrations.

Michaelis-Menten constant is the measurement of the affinity of an enzyme for its substrate. Km is numerically equal to the substrate concentrations or Km is equal to the concentration of the substrate, which gives half the maximum velocity, Vm/2



Rate of Reaction versus Substrate Concentration when $V = ½ V_m$

Coenzyme

A coenzyme is a substance that works with an enzyme to initiate or aid the function of the enzyme. It may be considered a helper molecule for a biochemical reaction.

Coenzymes are small, nonproteinaceous molecules that provide a transfer site for a functioning enzyme. They are intermediate carriers of an atom or group of atoms, allowing a reaction to occur. Coenzymes are not considered part of an enzyme's structure. They are sometimes referred to as cosubstrates. Coenzymes cannot function on their own and do require the presence of an enzyme. Some enzymes require several coenzymes and cofactors. The B vitamins serve as coenzymes essential for enzymes to form fats, carbohydrates and proteins. An example of a non-vitamin coenzyme is S-adenosyl methionine, which transfers a methyl group in bacteria as well as in eukaryotes and archaea.

Role of Coenzymes

Coenzymes play a role in the functions of cells. Reactions within the cells work to either break down nutrients or combine molecules for cellular activities that keep the cells alive. Enzymes speed up these reactions. Without enzymes, these reactions may not occur. Coenzymes, in turn, support the functions of enzymes. They loosely bind to enzymes to help them complete their activities. Coenzymes are nonprotein, organic molecules that facilitate the catalysis, or reaction, of its enzyme.

Cofactors

A cofactor is a non-protein chemical compound or metallic ion that is required for an enzyme's activity as a catalyst, a substance that increases the rate of a chemical reaction. Cofactors can be considered "helper molecules" that assist in biochemical transformations. The rates at which these happen are characterized by in an area of study called enzyme kinetics. Cofactors typically differ from ligands in that they often derive their function by remaining bound.

Cofactors can be divided into two types, either inorganic ions, or complex organic molecules called coenzymes. Coenzymes are mostly derived from vitamins and other organic essential nutrients in small amounts. Some enzymes or enzyme complexes require several cofactors. For example, the multienzyme complex pyruvate dehydrogenase and the citric acid cycle requires five organic cofactors and one metal ion.

Cofactors can be divided into two major groups:

1. **organic Cofactors**,
2. **inorganic cofactors**

Organic cofactors are sometimes further divided into *coenzymes* and *prosthetic groups*. The term coenzyme refers specifically to enzymes and, as such, to the functional properties of a protein. On the other hand, "prosthetic group" emphasizes the nature of the binding of a cofactor to a protein (tight or covalent) and, thus, refers to a structural property. Different sources give slightly different definitions of coenzymes, cofactors, and prosthetic groups.

inorganic cofactors: Some enzymes need assistance so that the catalytic process goes smoothly. Molecules, which can provide this assistance, are either cofactors or coenzymes.Coenzymes Are Cofactors -Coenzymes are one of two types of cofactors used by enzymes in these enzymatic reactions. The other types of cofactors are inorganic ions. Magnesium, calcium and potassium ions are commonly used with enzymes to speed up these reactions.

Function of cofactors

They are either loosely or tightly bond to the enzyme, which is often denatured when the cofactor is removed. These participate directly in the catalytic process, unlike coenzymes. Cofactors stabilize the enzyme or the substrate and assist directly in the reaction process.

In summary, they are inorganic stabilisers that assist directly in the catalytic reaction.

Some examples include:

 Mg2+ ions are cofactors in the DNA replication process that stabilizes the negatively charged DNA molecules.

Isozyme (or) Isoenzymes

The enzymes that occur in a number of differ-ent forms and differ from each other chemically, immunologically and electrophoretically are called "**Isoenzymes" or "isozymes**". Isozymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction.

Isoenzymes (isozymes) are multiple forms of the enzyme that have the same catalytic activity.

Although they have the same catalytic activity, they are physically distinct and differ in electrophoretic mobility and liability to inhibitors.

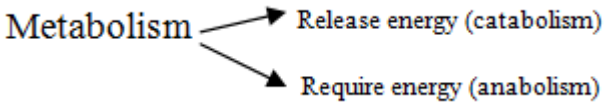
Iso- means the same and isoenzyme means the same enzyme.

Example of isoenzymes Many enzymes are present in isoenzyme form:

1. Lactate dehydrogenase
2. Creatine kinase
3. Acid phosphatase
4. Alkaline phosphatise

Lactate dehydrogenase (LDH) It is an enzyme that catalyzes the removal of 2 hydrogen atoms from lactic acid forming pyruvic acid.

Metabolism refers to the sum of all chemical reactions within a living organism. Chemical reactions either release or require energy. Metabolism can be viewed as an energy-balancing act .



Catabolism – enzyme-regulated chemical reactions that release energy. Complex organic compounds are broken down into simpler ones. These reactions are called catabolic or degradative reactions. They are generally hydrolytic reactions (reactions that use water and in which chemical bonds are broken), and they are exergonic (produce more energy than they consume). Ex. Cells break down sugars into CO_2 and H_2 O.

Anabolism – enzyme-regulated energy requiring reactions. The building of complex organic molecules from simpler ones. These reactions are called anabolic or biosynthetic and they are generally dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). Ex. Formation of proteins from amino acids, nucleic acids from nucleotides, polysaccharides from simple sugars)

These reactions generate the materials for growth. This coupling of energy requiring and energy-releasing reactions is made possible through the molecule **adenosine triphosphate (ATP).** ATP stores energy derived from catabolic reactions and releases it later to drive anabolic reactions and perform other cellular work.

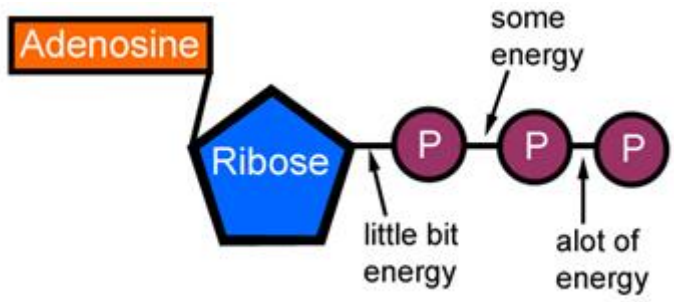


Fig. ATP molecule

ATP – an adenine, a ribose and 3 phosphate groups. When terminal phosphate group is split from ATP, ADP is formed, and energy is released to drive anabolic reactions.

ATP ⟶ ADP + Pi + energy

Then, energy from catabolic reactions is used to combine ADP and a P to resynthesize ATP.

ADP + Pi + energy ⟶ ATP

Anabolic reactions – coupled to ATP breakdown

Catabolic reactions – ATP synthesis.

ENZYMES:

• Substances that can speed up a chemical reaction without being permanently altered themselves are called **catalysts**.

• In living cells, enzymes serve as biological catalysts.

• Enzymes are specific and act on specific substances called the enzyme **substrate(s)** and each catalyzes only one reaction.

Ex. Sucrose is the substrate of the enzyme sucrose, which catalyzes the hydrolysis of sucrose to glucose and fructose.

Enzyme specificity and efficiency:

Enzymes are large globular proteins that range in MW from about 10,000 to several million. Each enzyme has a characteristic three-dimensional shape with a specific surface configuration as a result of its primary, secondary and tertiary structures. This enables it to find the correct substrate from among the large number of diverse molecules in the cell. Enzymes are extremely efficient. Under optimum conditions can catalyze reactions at rates 10^8 to 10^{10} times higher than those of comparable reactions without enzymes. **Turnover number** (maximum number of substrate molecules an enzyme molecule converts to product each second) is between 1 and 10,000 and can be high as 500,000.

Enzyme components:

• Some enzymes consist entirely of proteins.

• Most consist of both a protein portion called an **apoenzyme** and a nonprotein component called a **cofactor**.

• Ions of iron, zinc, magnesium or calcium are examples of cofactors. If the **cofactor** is an **organic molecule**, it is called a **coenzyme**.

• Together, the apoenzyme and cofactor form a **holoenzyme**, or whole active enzyme. If the cofactor is removed, the apoenzyme will not function.

• Coenzymes assist the enzyme by accepting atoms removed from the substrate or by donating atoms required by the substrate. Some act as electron carriers, removing electrons from the substrate and donating them to other molecules in subsequent reactions.

• Many coenzymes are derived from vitamins. Two of the most important coenzymes in cellular metabolism are

Nicotinamide adenine dinucleotide (NAD^+)

Nicotinamide adenine dicucleotide phosphate ($NADP^+$)

These contain derivatives of B vitamin nicotinic acid (niacin)

NAD^+ - involved in catabolic (energy-yielding reactions)

$NADP^+$ - involved in anabolic (energy-requiring reactions)

• Flavin coenzymes, such as flavin mononucleotide **(FMN)** and flavin adenine dinucleotide **(FAD),** contains derivatives of the B vitamin riboflavin and are also electron carriers.

• Coenzyme A **(CoA)** contains derivatives of pantothenic acid, another B vitamin. This plays an important role in the synthesis and breakdown of fats and in a series of oxidizing reactions called the Krebs cycle.

• Some cofactors are metal ions, including Fe, Cu, Mg, Mn, Zn, Ca and Co. They form a bridge between the enzyme and a substrate. Ex. Mg^{2+} is required by many phosphorylating enzymes (enzymes that transfer a phosphate group from ATP to another substrate).

Energy production:

There are two general aspects of energy production; the concept of oxidation-reduction and the mechanism of ATP generation.

Oxidation – reduction reactions:

Oxidation – is removal of electron from an atom or molecule, a reaction that often produces energy.

Reduction – is addition of one or more electrons to an atom or molecule.

Oxidation and reductions reactions are always coupled. The pairing of these reactions is called oxidation-reduction or redox reactions. Most biological oxidation reactions involve the loss of hydrogen atoms, they are also dehydrogenation. Hydrogen atom contains both electrons and protons and in cellular oxidations, electrons and protons are removed at the same time. An organic molecule is oxidized by the loss of two hydrogen atoms, and a molecule of NAD^+ is reduced by accepting two electrons and one proton. One proton is left over and is released into the surrounding medium. The reduced coenzyme contains more energy than NAD^+. This energy can be used to generate ATP in later reactions.

Cells use oxidation- reduction (biological) reactions in catabolism to extract energy from nutrient molecules. Ex. Cell oxidizes a molecule of glucose to CO_2 and H_2O. The energy in the glucose molecule is removed in stepwise manner and ultimately is trapped by ATP, which can then serve as an energy source for energy requiring reactions. Thus glucose is a valuable nutrient for organisms.

The generation of ATP:

Much of the energy released during oxidation – reduction reactions is trapped within the cell by the formation of ATP. A phosphate group is added to ADP with the input of energy to form ATP. Addition of a phosphate to a chemical compound is called **phosphorylation**. Organisms use three mechanisms of phosphorylation to generate ATP from ADP.

Substrate – level phosphorylation: ATP is generated when a high energy phosphate is directly transferred from a phosphorylated compound (a substrate) to ADP. Generally the phosphate has acquired its energy during an earlier reaction in which the substrate itself was oxidized.

$$\textbf{C-C-C}\sim\textbf{P} + \textbf{ADP} \longrightarrow \textbf{C-C-C} + \textbf{ATP}$$

Oxidative phosphorylation: Electrons are transferred from organic compounds to one group of electron carriers (usually to NAD^+ and FAD). Then, the electrons are passed through a series of different electron carriers to molecules of O_2 or other oxidized inorganic and organic molecules. This process occurs in the plasma membrane of prokaryotes and in the inner mitochondrial membrane of eukaryotes. The sequence of electron carriers used in oxidative phosphorylation is called an electron transport chain. The transfer of electrons from one electron carrier to the next releases energy, some of which is used to generate ATP from ADP through a process called **chemiosmosis**.

Photophosphorylation: Occurs only in photosynthetic cells which contain light-trapping pigments such as chlorophylls. In photosynthesis, organic molecules, especially sugars, are synthesized with the energy of light from the energy-poor building blocks CO_2 and H_2O. Photophosphorylation starts this process by converting light energy to the chemical energy of ATP and NADPH, which in turn, are used to synthesize organic molecules. As in oxidative phosphorylation, an electron transport chain is involved.

All microbial metabolisms can be arranged according to three principles:

1. How the organism obtains carbon for synthesizing cell mass?
   - **autotrophic** – carbon is obtained from carbon dioxide (CO_2)
   - **heterotrophic** – carbon is obtained from organic compounds
   - **mixotrophic** – carbon is obtained from both organic compounds and by fixing carbon dioxide.

2. How the organism obtains reducing equivalents used either in energy conservation or in biosynthetic reactions:

- **lithotrophic** – reducing equivalents are obtained from inorganic compounds
- **organotrophic** – reducing equivalents are obtained from organic compounds

3. How the organism obtains energy for living and growing:

- **chemotrophic** – energy is obtained from external chemical compounds
- **phototrophic** – energy is obtained from light.

- **chemolithoautotrophs** obtain energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide. Examples: Nitrifying bacteria, Sulfur-oxidizing bacteria, Iron-oxidizing bacteria, Knallgas-bacteria

- **photolithoautotrophs** obtain energy from light and carbon from the fixation of carbon dioxide, using reducing equivalents from inorganic compounds. Examples: Cyanobacteria (water (H_2O) as reducing equivalent donor), Chlorobiaceae, Chromatiaceae (hydrogen sulfide (H_2S) as reducing equivalent donor), *Chloroflexus* (hydrogen (H_2) as reducing equivalent donor)

- **chemolithoheterotrophs** obtain energy from the oxidation of inorganic compounds, but cannot fix carbon dioxide (CO_2). Examples: some *Thiobacilus*, some *Beggiatoa*, some *Nitrobacter* spp., *Wolinella* (with H_2 as reducing equivalent donor), some Knallgas-bacteria, some sulfate-reducing bacteria

- **chemoorganoheterotrophs** obtain energy, carbon, and reducing equivalents for biosynthetic reactions from organic compounds. Examples: most bacteria, e. g. *Escherichia coli*, *Bacillus* spp., *Actinobacteria*

- **photoorganoheterotrophs** obtain energy from light, carbon and reducing equivalents for biosynthetic reactions from organic compounds. Some species are strictly heterotrophic, many others can also fix carbon dioxide and are mixotrophic. Examples: *Rhodobacter*, *Rhodopseudomonas*, *Rhodospirillum*, *Rhodomicrobium*, *Rhodocyclus*, *Heliobacterium*, *Chloroflexus* (alternatively to photolithoautotrophy with hydrogen).

The Metabolism of Microbes

All cells require the constant input and expenditure of some form of usable energy. Metabolic pathways use many enzymes and coenzymes to extract chemical energy present in nutrient fuels (like the sugar glucose) and apply that energy towards useful work in the cell (cell maintenance, growth, and development).

Metabolism refers to all the chemical reactions that take place in a cell. It involves two principle types of reactions: anabolic reactions and catabolic reactions.

(i) Anabolism is a building up reaction and generally requires energy (i.e. is endergonic). Examples: construction of a new cell, assimilation of nutrients. During anabolic reactions small molecules become larger more complex molecules (e.g. amino acids become proteins).

(ii) Catabolism includes all reactions that result in the breakdown of large organic molecules into simpler ones (usually involving the release of energy, i.e. are exergonic). Example: Glycolysis is the catabolic breakdown of glucose that releases energy.

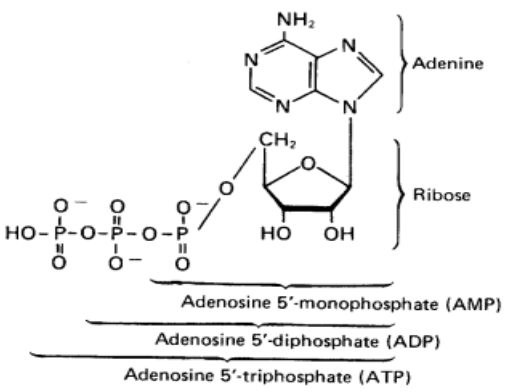
Energy generated by catabolic reactions is used to power anabolic reactions. When bonds break, energy is released. It is similar to burning wood, i.e. as the wood breaks down, heat energy is released and that heat energy can be used to do work. In biochemical reactions the energy released from breaking bonds can be stored as ATP (adenosine triphosphate).

Chemical principles of metabolism

A part of the energy released from oxidation of foodstuffs and from light gets stored in a molecule, is known as adenosine triphosphate (ATP). It, then, transfers this energy to reactions that require energy. ATP functions as a carrier of energy in all living organisms including bacteria,fungi, plants and animals. In other words, every cell stores and uses energy, biochemically, through ATP. Therefore, it is considered a universal currency of biological energy.

ATP was discovered in 1929 by Karl Lohmann.It was proposed to be the main energytransfer molecules in the cell by Fritz Albert Lipmann in 1941. ATP is a nucleotide consisting of apurine base (adenine), a pentose sugar (ribose) and three phosphate groups (triphosphate unit). Adenine is attached to the 1’carbon atom and the phosphate groups are

attached at the 5'carbon atom of the ribose. ATP, in its active form, exists as a complex of ATP with Mg2+ or Mn2+ ions.



The structure of adenosine triphosphate (ATP). ATP is a typical nucleotide consisting of an adenine ring, a ribose, and three phosphate groups.

Heterotrophic Metabolism

Heterotrophic bacteria, which include all pathogens, obtain energy from oxidation of organic compounds. Carbohydrates (particularly glucose), lipids, and protein are the most commonly oxidized compounds. Biologic oxidation of these organic compounds by bacteria results in synthesis of ATP as the chemical energy source. This process also permits generation of simpler organic compounds (precursor molecules) needed by the bacteria cell for biosynthetic or assimilatory reactions.

The Krebs cycle intermediate compounds serve as precursor molecules (building blocks) for the energy-requiring biosynthesis of complex organic compounds in bacteria. Degradation reactions that simultaneously produce energy and generate precursor molecules for the biosynthesis of new cellular constituents are called amphibolic.

All heterotrophic bacteria require preformed organic compounds. These carbon- and nitrogen-containing compounds are growth substrates, which are used aerobically or anaerobically to generate reducing equivalents (e.g., reduced nicotinamide adenine dinucleotide; NADH + H^+); these reducing equivalents in turn are chemical energy sources for all biologic oxidative and fermentative systems. Heterotrophs are the most commonly studied bacteria; they grow readily in media containing carbohydrates, proteins, or other complex nutrients such as blood. Also, growth media may be enriched by the addition of other naturally occurring compounds such as milk (to study lactic acid bacteria) or hydrocarbons (to study hydrocarbon-oxidizing organisms).

Metabolism of carbohydrates

Most microorganisms oxidize carbohydrates as their primary source of cellular energy. Glucose is the most common carbohydrate energy source used by cells. To produce energy from glucose microorganisms use two general processes: cellular respiration and fermentation. Anaerobic respiration is another mode where the final electron acceptor is an inorganic substance other than oxygen.

Catabolism/Oxidation of carbohydrates or Aerobic respiration of carbohydrates:

-- Most efficient way to extract energy from glucose. Occurs in three principal stages:

1. **Glycolysis**
2. **Kreb Cycle**
3. **Electron transport chain**

Glycolysis – Oxidation of glucose to pyruvic acid with the production of some ATP and energy containing NADH.

Krebs cycle – Oxidation of acetyl (a derivative of pyruvic acid) to Co_2, with the production of some ATP, energy containing NADH, and another reduced electron carrier, $FADH_2$.

Electron Transport chain – NADH and $FADH_2$ are oxidized, contributing the electrons, they have carried from the substrate to a 'cascade' of oxidation-reduction reactions involving a series of additional electron carriers. Energy from these reactions is used to generate a considerable amount of ATP. In respiration, most of the ATP is generated in this step.

Fermentation: Initial stage is also glycolysis which produces pyruvic acid. But pyruvic acid is converted into one or more different products, depending on the type of cell. These products might include alcohol and lactic acid. Unlike respiration, there is no Krebs cycle or electron transport chain. Accordingly, the ATP yield is also much lower.

Glycolysis Or Embden-Meyerhof (EMP) pathway:

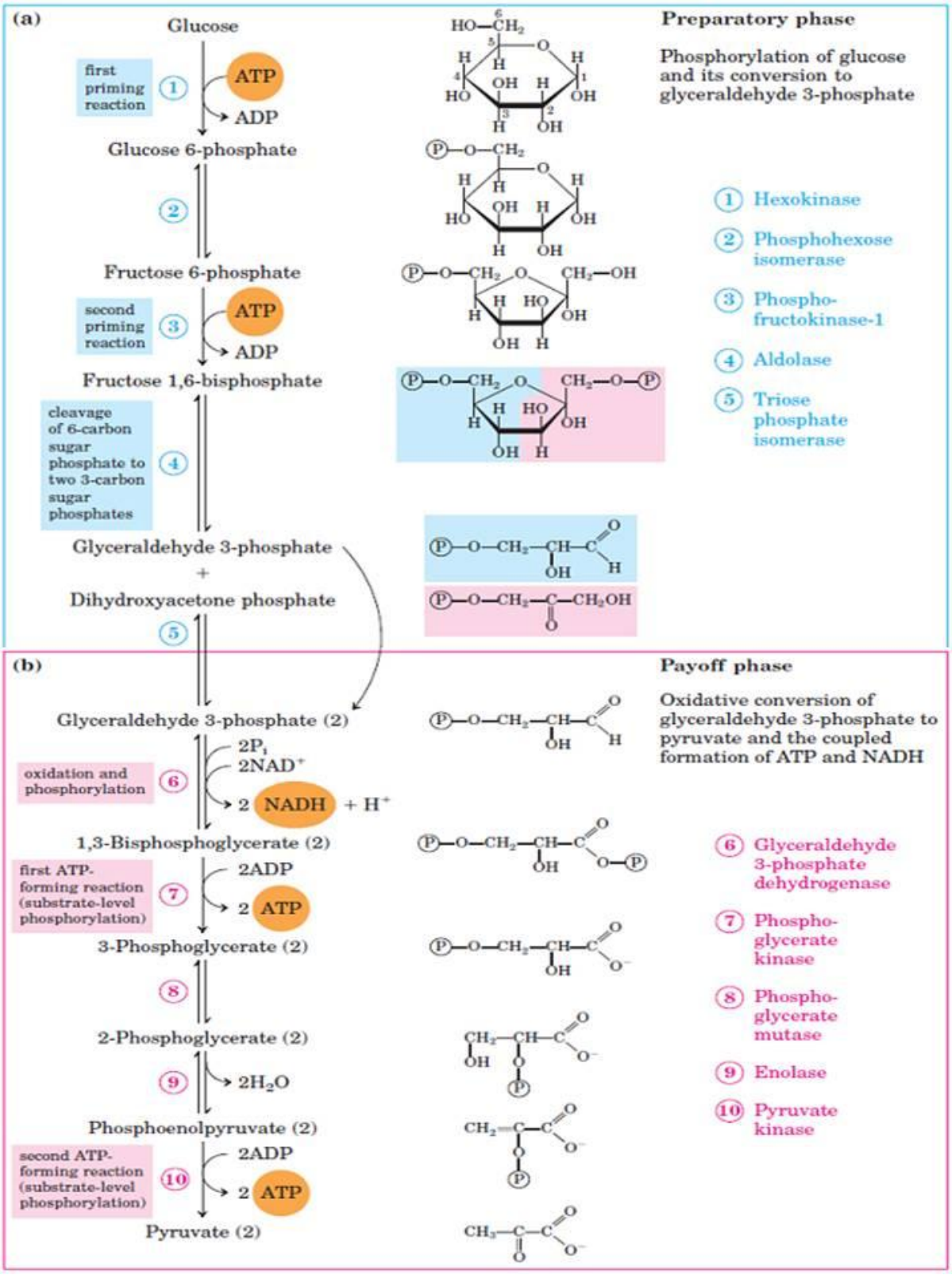
In **glycolysis** (from the Greek *glykys,* meaning "sweet,"and *lysis,* meaning "splitting"), a molecule of glucose is degraded in a series of enzyme-catalyzed reactions to yield two molecules of the three-carbon compound pyruvate. During glycolysis NAD^+ is reduced to

NADH and there is a net production of 2 ATP molecules by substrate level phosphorylation. Glycolysis does not require oxygen and can occur whether present or not.

Reactions in glycolytic pathway

Glycolysis involves 10 enzymatic reactions:

1. The phosphorylation of glucose at position 6 by hexokinase,
2. The isomerization of glucose-6-phosphate to fructose-6-phosphate by phosphohexose isomerase,
3. The phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate by phosphofructokinase,
4. The cleavage of fructose-1,6-bisphosphate by aldolase. This yields two different products, dihydroxyacetone phosphate and glyceraldehyde-3-phos phate,
5. The isomerization of dihydroxyacetone phosphate to a second molecule of glyceraldehyde phosphate by triose phosphate isomerase,
6. The dehydrogenation and concomitant phosphorylation of glyceralde- hyde-3-phosphate to 1,3-bis-phosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase,
7. The transfer of the 1-phosphate group from 1,3-bis-phosphoglycerate to ADP by phosphoglycerate kinase, which yields ATP and 3-phosphoglycerate,
8. The isomerization of 3-phosphoglycerate to 2-phosphoglycerate by phos phoglycerate mutase,
9. The dehydration of 2-phosphoglycerate to phosphoenolpyruvate by eno lase.
10. The transfer of the phosphate group from phosphoenolpyruvate to ADP by pyruvate kinase, to yield a second molecule of ATP.


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2P$_i$
2NAD$^+$
2 NADH + H$^+$
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H$_2$O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

Overall reaction of glycolysis

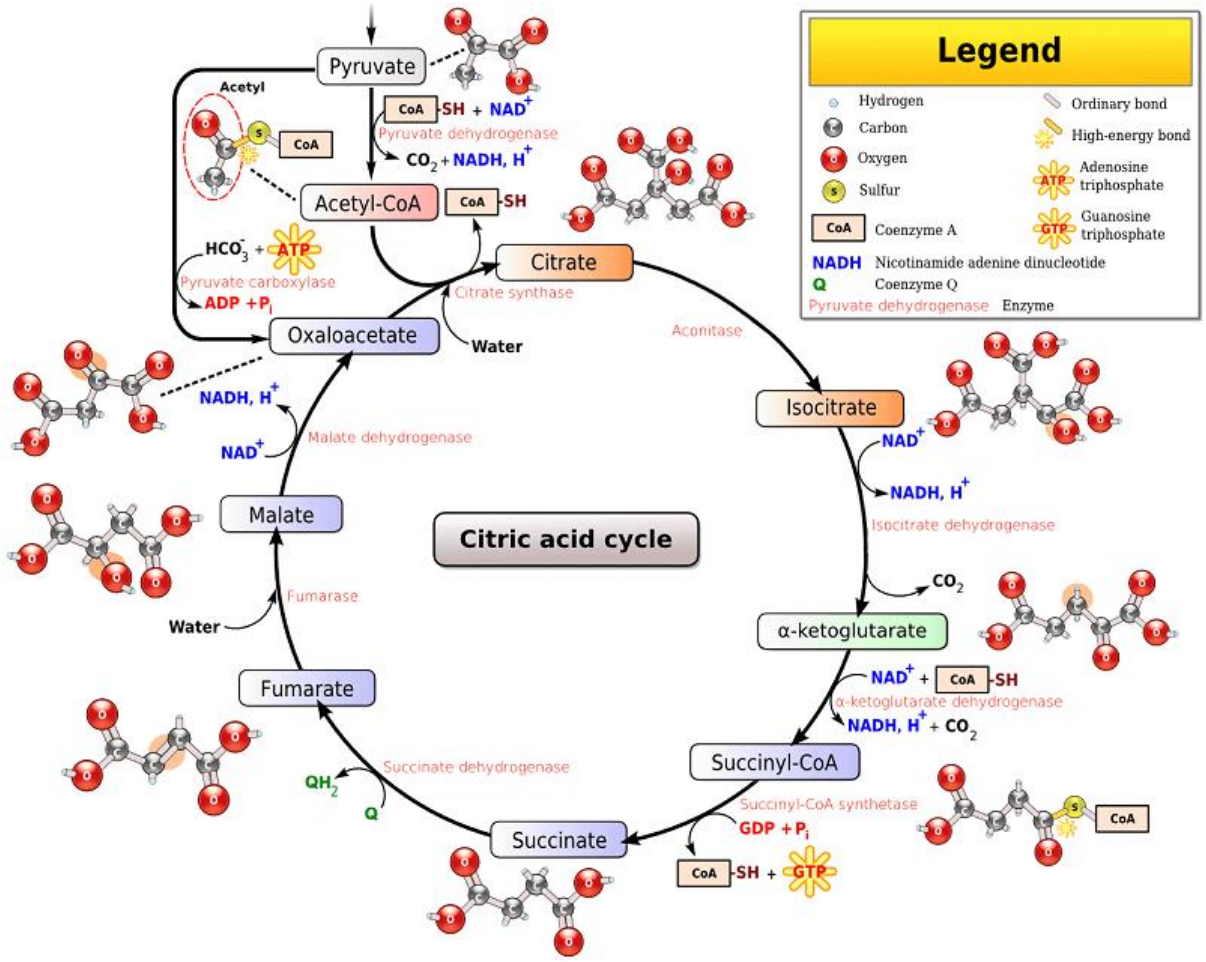
$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \longrightarrow 2\ \text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

Because 2 moleucles of ATP were needed to get glycolysis started and four molecules of ATP are generated by the process, there is a net gain of two molecules of ATP for each molecule of glucose that is oxidised.

The Krebs cycle /Citric Acid Cycle/ Tricarboxylic Acid Cycle

The pyruvate produced by glycolysis is oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and are not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways.

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion . Bacteria also use the TCA cycle to generate energy, but since they lack mitochondria, the reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the plasma membrane rather than the inner membrane of the mitochondrion.



- • Pyruvic acid, the product of glycolysis, cannot enter the Krebs cycle directly. In a preparatory step; it must lose one molecule of Co_2 and become a two-carbon compound. This process is called **decarboxylation**. The two carbon compound called an acetyl group, attaches to Coenzyme a through a high-energy bond, the resulting complex is known as Acetyl Coenzyme A. During this reaction, pyruvic acid is also oxidized and NAD^+ is reduced to NADH.

  • Oxidation of one glucose molecule produces 2 molecules of pyruvic acid, so for each molecule of glucose, 2 molecules of Co_2 are released in the preparatory step, 2 molecules of NADH are produced, and 2 molecules of Acetyl Coenzyme A are formed.

  • As Acetyl coenzyme A enters the Krebs cycle, CoA detaches from the acetyl group. The two carbon acetyl group combines with a four carbon compound called oxaloacetic acid to form six carbon compound, called citric acid. This synthesis reaction requires energy, which is provided by the cleavage of the high energy bond between the acetyl group and CoA. The formation of citric acid is the first step in the Krebs cycle.

  • Two decorboxylation reactions take place in the Krebs cycle while converting Isocitric acid to α – Ketoglutaric acid and this to succinyl CoA.

• Altogether 3 decarboxylation reactions take place and hence all three carbon atoms in pyruvic acid are eventually released as Co_2 by the Krebs cycle. This represents the conversion to Co_2 by all 6 carbon atoms contained in the original glucose molecule.

• Oxidation-reduction reactions also occurs, where NAD^+ and FAD picks up hydrogen atoms to be reduced to NADH and $FADH_2$.

• On the whole, for every two molecules of acetyl CoA that enter the cycle, 4 molecules of Co_2 and 6 for pyruvic acid are liberated by decorboxylation, 6/8 moelucles of NADH and 2 moleucles of $FADH_2$ are produced by oxidation-reduction reactions, and two molecules of ATP are generated by substrate- level phosphorylation. Many of the intermediates in the Krebs cycle also play a role in other pathways, especially in amino acid biosynthesis.

• Reduced coenzymes NADH and $FADH_2$ are the important products of the Krebs cycle because they contain most of the energy originally stored in glucose. During the next phase of respiration, a series of reductions indirectly transfers the energy stored in those coenzymes to ATP. These reactions are collectively called Electron transport chain.

The Electron Transport Chain:

- • Consists of a sequence of carrier molecules that are capable of oxidation and reduction.

  • As electrons are passed through the chain, there is a stepwise release of energy, used to drive the chemiosmotic generation of ATP.

  • In eukaryotic cells, it is contained in the inner membrane of mitochondria.

  • In prokaryotes, it is found in the plasma membrane.

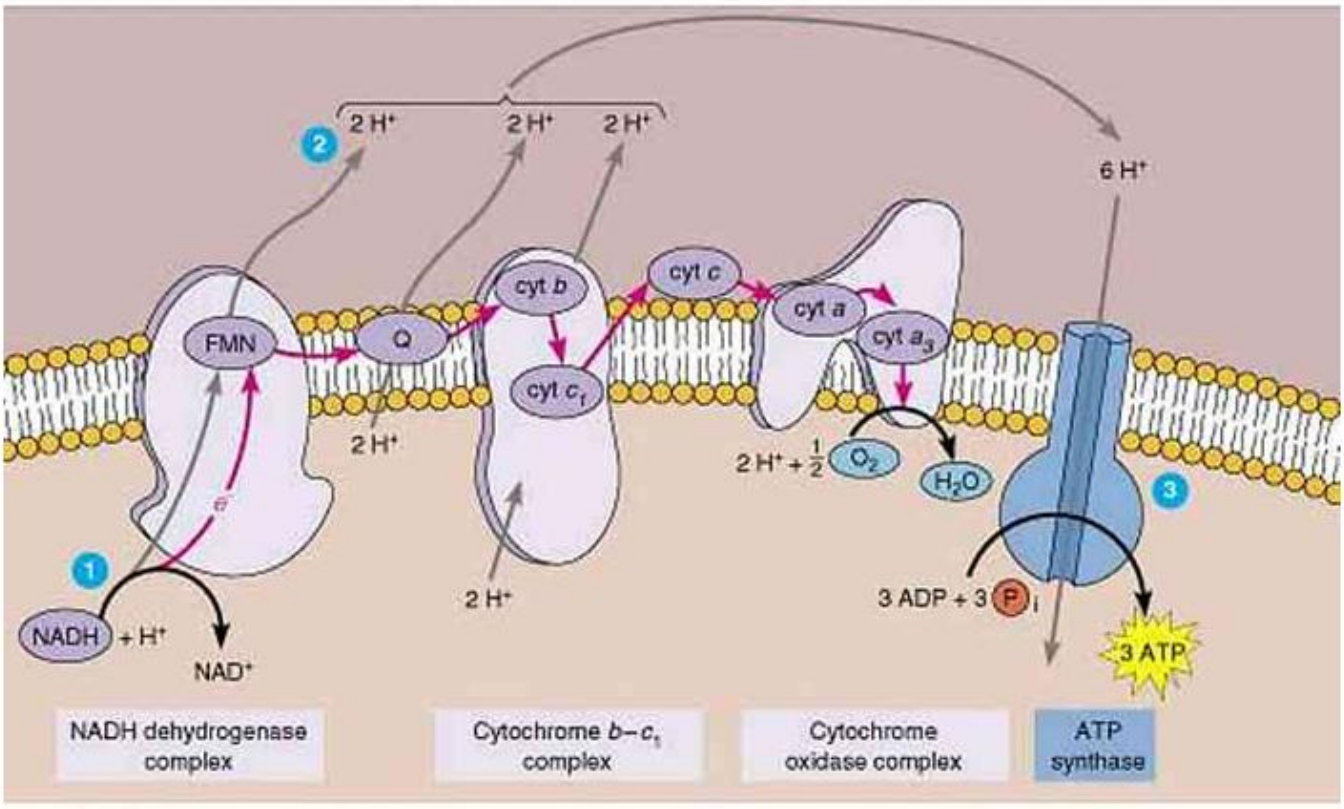
  Three classes of carrier molecules are involved:

  1. Flavoproteins – these contain flavin, a coenzyme derived from riboflavin (Vitamin B_2). One important flavin coenzyme is flavin mononucleotide (FMN).

  2. Cytochromes – proteins with an iron-containing group capable of existing alternately as a reduced form (Fe^{2+}) and an oxidized form (Fe^{3+}). The cytochormes include cytochrome b, C_1, a, a_3.

  3. Ubiquinones or Coenzyme Q – these are small non-protein carriers.

- Electron transport chains of bacteria are somewhat diverse, and the particular carriers and the order in which they functions may differ from those of other bacteria and from those of eukaryotic mitochondrial systems. Much is known about the electron transport chain in the mitochondria of eukaryotic cells.



- 1. Transfer of high energy electrons from NADH to FMN, the first carrier in the chain. This transfer involves at the passage of a hydrogen atom with 2^{e-} to FMN, which then pick up an additional H^+ from the surrounding aqueous medium. NADH is oxidised to NAD^+ and FMN reduced to $FMNH_2$.

2. $FMNH_2$ passes $2H^+$ to the other side of the mitochondrial membrane and passes 2^{e-} to Q. As a result $FMNH_2$ is oxidized to FMN. Q picks up an additional $2H^+$ from the medium and releases it on the other side of the membrane.

3. Electrons are passed successively from Q to Cyt b, cyt c_1, cyt c, cyt a and cyt a_3. Each cytochrome in the chain is reduced as it picks up e-and is oxidised as it gives up electrons. The last cyt a_3 passes it electrons to molecular O_2, which becomes negatively charged and then picks up protons from the medium to form H_2O.

- • $FADH_2$ adds its electrons to the electron transport chain at a lower level than NADH. Because of this, the electron transport chain produces about one-third less energy for ATP generation when $FADH_2$ donates electrons than when NADH is involved.

• FMN and Q accept and release protons as well as electrons and other carrier cytochromes transfer only electrons.

• Electron flow down the chain is accompanied at several points by the active transport (Pumping) of protons from the matrix side of the inner mitochondrial membrane to the opposite side of the membrane. The result is build up of protons on one side of the membrane, which provides energy for the generation of ATP by the chemiosmotic mechanism.

Chemiosmotic mechanism of ATP generation:

- • Mechanism of ATP synthesis using the electron transport chain is called chemiosmosis.

• Substances diffuse passively across membranes from areas of high concentration to areas of low concentration, this diffusion yields energy. In chemiosmosis, the energy released when a substance moves along a gradient is used to synthesize ATP.

1. As energetic electrons from NADH (or chlorophyll) pass down the electron transport chain, some of the carriers in the chain pump actively transport – protons across the membrane. Such carrier molecules are called proton pumps.

2. The phospholipid membrane is normally impermeable to protons, so this one-directional pumping establishes a proton gradient. The excess H^+ on one side of the membrane makes that side positively charged compared with the other side. The resulting electrochemical gradient has potential energy, called the proton motive force.

3. The protons on one side of the membrane can diffuse across the membrane only through special protein channels that contain an enzyme called adenosine triphosphate (ATP synthase). When this flow occurs, energy is released and is used by the enzyme to synthesize ATP from ADP and P_i.

• Electron transport chain also operates in photophosphorylation and is located in the thylakoid membrane of cyanobacteria and eukaryotic chloroplasts.

Summary of Aerobic respiration:

- • Electron transport chain regenerates NAD^+ and FAD^+ which can be used again in glycolysis and Krebs cycle.

• Various electron transfers in the electron transport chain generates about 34 molecules of ATP from each molecule of glucose oxidized, 10 NADH and 2 $FADH_2$.

• A total of 38 ATP molecules can be generated from one molecule of glucose in prokaryotes.

• A total of 36 molecules of ATP are produced in eukaryotes. Some energy is lost when electrons are shuttled across the mitochondrial membranes that separate glycolysis (in the cytoplasm) from the electron transport chain. No such separation exists in prokaryotes.

$$C_6H_{12}O_6 + 6\ CO_2 + 38ADP + 38\ P_i \rightarrow 6CO_2 + 6H_2O + 38\ ATP$$

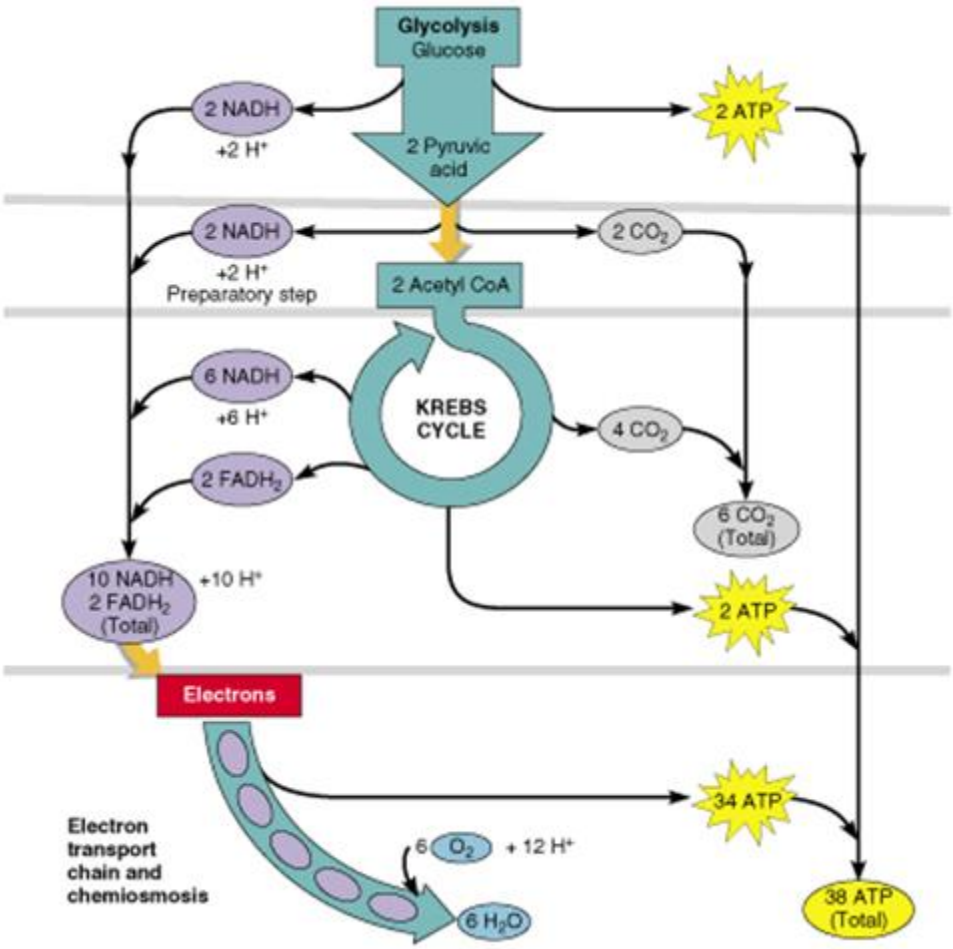


Fig. Generation of ATPs and NADH/$FADH_2$ during Aerobic Respiration

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PHOTOSYNTHESIS

Photosynthesis is the use of light as a source of energy for growth, more specifically the conversion of light energy into chemical energy in the form of ATP. Prokaryotes that can convert light energy into chemical energy include the photosynthetic cyanobacteria, the purple and green bacteria, and the "halobacteria" (actually archaea). The cyanobacteria conduct plant photosynthesis, called **oxygenic photosynthesis**; the purple and green bacteria conduct bacterial photosynthesis or **anoxygenic photosynthesis**; the extreme halophilic archaea use a type of **nonphotosynthetic photophosphorylation** mediated by a pigment, bacteriorhodopsin, to transform light energy into ATP.

Net equation:

$6CO_2 + 12H_2O + LightEnergy \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_20$

Photosynthetic reactions divided into two stages:

- • **Light reaction** - light energy absorbed & converted to chemical energy (ATP, NADPH)

  • **Dark reaction-** carbohydrates made from CO_2 using energy stored in ATP & NADPH

Types of bacterial photosynthesis

1. Oxygenic Photosynthesis

• Occurs in cyanobacteria and prochlorophytes

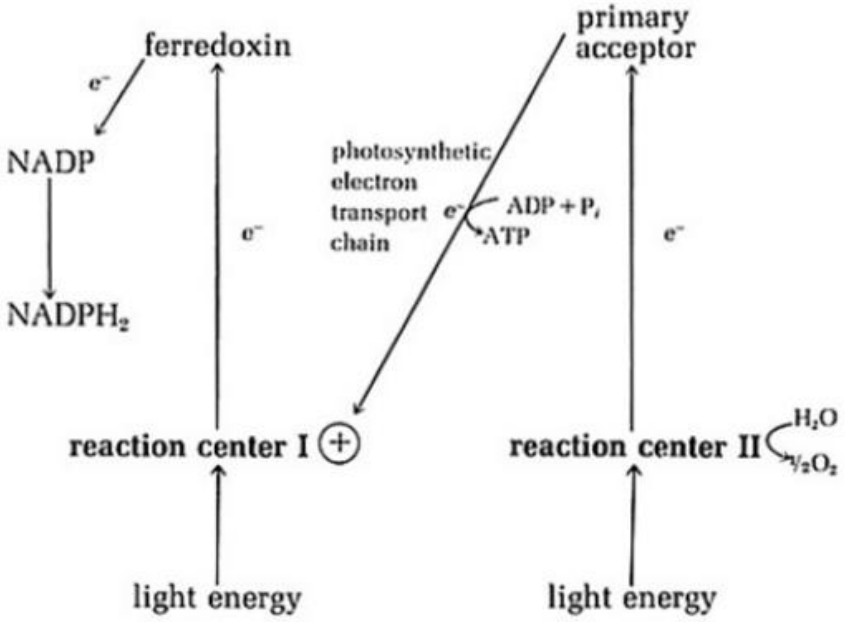
• Synthesis of carbohydrates results in release of molecular O_2 and removal of CO_2 from atmoshphere.

• Occurs in lamellae which house thylakoids containing chlorophyll a/b and phycobilisomes pigments which gather light energy

• Uses two photosystems (PS):

- PS II- generates a proton-motive force for making ATP.

- PS I- generates low potential electrons for reducing power.



Electron flow in oxygenic photosynthesis.

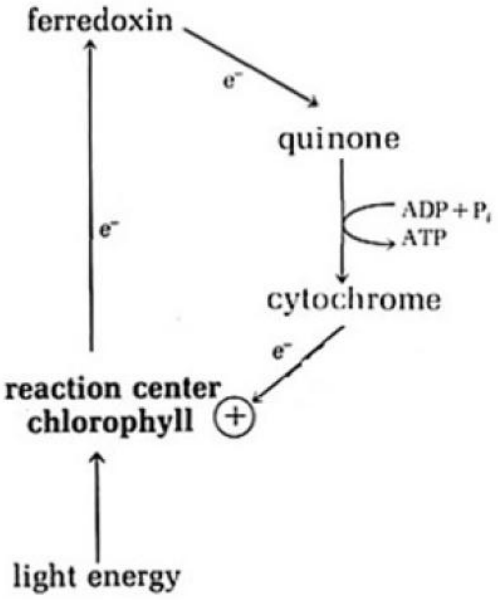
2. Anoxygenic Photosynthesis

• Uses light energy to create organic compounds, and sulfur or fumarate compounds instead of O_2.

• Occurs in purple bacteria, green sulfur bacteria, green gliding bacteria and heliobacteria.

• Uses bacteriochlorophyll pigments instead of chlorophyll.

• Uses one photosystem (PS I) to generate ATP in “cyclic” manner.



Cyclical Flow of Electrons during Anoxygenic Photosynthesis.

Light Reaction:

The Light Reactions depend upon the presence of chlorophyll, the primary lightharvesting pigment in the membrane of photosynthetic organisms. The functional components of the photochemical system are light harvesting pigments, a membrane electron transport system, and an ATPase enzyme. The photosynthetic electron transport system of is fundamentally similar to a respiratory ETS, except that there is a low redox electron acceptor (for example ferredoxin) at the top (low redox end) of the electron transport chain, that is first reduced by the electron displaced from chlorophyll.

Structure of Photosynthetic Pigments:

There are several types of pigments distributed among various phototrophic organisms.

- ✓ **Chlorophyll** is the primary light-harvesting pigment in all photosynthetic organisms. Chlorophyll is a tetrapyrrole which contains magnesium at the center of the porphyrin ring. It contains a long hydrophobic side chain that associates with the photosynthetic membrane. Cyanobacteria have **chlorophyll a**, the same as plants and algae. The chlorophylls of the purple and green bacteria, called **bacteriochlorophylls** are chemically different than chlorophyll a in their substituent side chains. This is reflected in their light absorption spectra. Chlorophyll a absorbs light in two regions of the spectrum, one around 450nm and the other between 650 -750nm; bacterial chlorophylls absorb from 800-1000nm in the far red region of the spectrum.

- ✓ **Carotenoids** are always associated with the photosynthetic apparatus. They function as **secondary light-harvesting pigments** , absorbing light in the blue-green spectral region between 400-550 nm. Carotenoids transfer energy to chlorophyll, at near 100 percent efficiency, from wave lengths of light that are missed by chlorophyll. In addition, carotenoids have an indispensable function to protect the photosynthetic apparatus from photooxidative damage. Carotenoids have long hydrocarbon side chains in a conjugated double bond system. Carotenoids "quench" the powerful oxygen radical, singlet oxygen, which is invariably produced in reactions between chlorophyll and O_2 (molecular oxygen). Some non-photosynthetic bacterial pathogens, i.e., *Staphylococcus aureus*, produce carotenoids that protect the cells from lethal oxidations by singlet oxygen in phagocytes.
- ✓ **Phycobiliproteins** are the major light harvesting pigments of the cyanobacteria. They also occur in some groups of algae. They may be red or blue, absorbing light in the middle of the spectrum between 550 and 650nm. Phycobiliproteins consist of proteins that contain covalently-bound linear tetrapyrroles (**phycobilins**). They are contained in granules called **phycobilisomes** that are closely associated with the photosynthetic apparatus. Being closely linked to chlorophyll they can efficiently transfer light energy to chlorophyll at the reaction center.

All phototrophic bacteria are capable of performing cyclic photophosphorylation this universal mechanism of cyclic photophosphorylation is referred to as **Photosystem I**. Bacterial photosynthesis uses only Photosystem I (PSI), but the more evolved cyanobacteria, as well as algae and plants, have an additional light-harvesting system called Photosystem II (PSII). **Photosystem II** is used to reduce Photosystem I when electrons are withdrawn from PSI for CO_2 fixation.

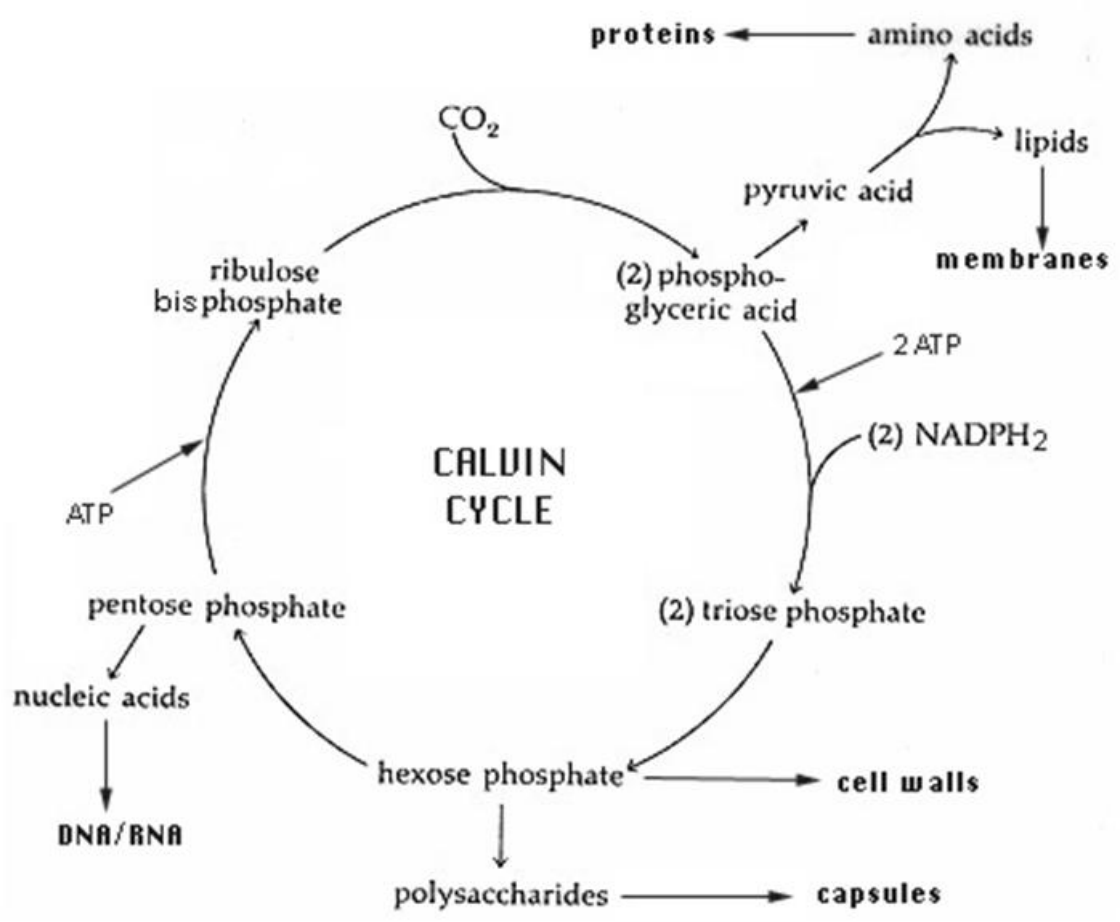
Dark reaction

The use of **RUBP carboxylase and the Calvin cycle** is the most common mechanism for CO_2 fixation among autotrophs. Indeed, RUBP carboxylase is said to be the most abundant enzyme on the planet (nitrogenase, which fixes N_2 is second most abundant). This is the only mechanism of autotrophic CO_2 fixation among eucaryotes, and it is used, as well, by all cyanobacteria and purple bacteria. Lithoautotrophic bacteria also use this pathway. But the green bacteria and the methanogens, as well as a few isolated groups of procaryotes, have alternative mechanisms of autotrophic CO_2 fixation and do not possess RUBP carboxylase.

RUBP carboxylase (**ribulose bisphosphate carboxylase**) uses ribulose bisphosphate (RUBP) and CO_2 as co-substrates. In a complicated reaction the CO_2 is "fixed" by addition to

the RUBP, which is immediately cleaved into two molecules of 3-phosphoglyceric acid (PGA). The fixed CO_2 winds up in the -COO group of one of the PGA molecules. Actually, this is the reaction which initiates the Calvin cycle.

The Calvin cycle is concerned with the conversion of PGA to intermediates in glycolysis that can be used for biosynthesis, and with the regeneration of RUBP, the substrate that drives the cycle. After the initial fixation of CO_2, 2 PGA are reduced and combined to form hexose-phosphate by reactions which are essentially the reverse of the oxidative Embden-Meyerhof pathway. The hexose phosphate is converted to pentose-phosphate, which is phosphorylated to regenerate RUBP. An important function of the Calvin cycle is to provide the organic precursors for the biosynthesis of cell material. Intermediates must be constantly withdrawn from the Calvin cycle in order to make cell material. In this regard, the Calvin cycle is an anabolic pathway. The fixation of CO_2 to the level of glucose ($C_6H_{12}O_6$) requires 18 ATP and 12 $NADPH_2$.



The Calvin cycle and its relationship to the synthesis of cell materials.

Most of the phototrophic prokaryotes are autotrophs, which mean that they are able to fix CO2 as a sole source of carbon for growth. Just as the oxidation of organic material yields energy, electrons and CO2 , in order to build up CO2 to the level of cell material (CH2O),

energy (ATP) and electrons (reducing power) are required. The overall reaction for the fixation of CO2 in the Calvin cycle is CO2 + 3ATP + 2NADPH2 · CH2O + 2ADP + 2Pi + 2NADP. The light reactions operate to produce ATP to provide energy for the dark reactions of CO2 fixation. The dark reactions also need reductant (electrons). Usually the provision of electrons is in some way connected to the light reactions.

Protein and Amino acid Catabolism

Some bacteria and fungi particularly pathogenic, food spoilage and soil microorganisms can use proteins as their source of carbon and energy.

1. 1. Proteases are enzymes that break down proteins into amino acids

2. Amino acids are deaminated, and then enter the Kreb's Cycle.

Intact proteins cannot cross bacterial plasma membrane, so bacteria must produce extracellular enzymes called proteases and peptidases that break down the proteins into amino acids, which can enter the cell. Many of the amino acids are used in building bacterial proteins, but some may also be broken down for energy. If this is the way amino acids are used, they are broken down to some form that can enter the Kreb's cycle. These reactions include:

1. 1. **Deamination or Transamination** —the amino group is removed or transferred, or converted to an ammonium ion, and excreted. The remaining organic acid (the part of the amino acid molecule that is left after the amino group is removed) can enter the Kreb's cycle.

2. **Decarboxylation** —the ---COOH group is removed.

3. **Dehydrogenation** —a hydrogen is removed.

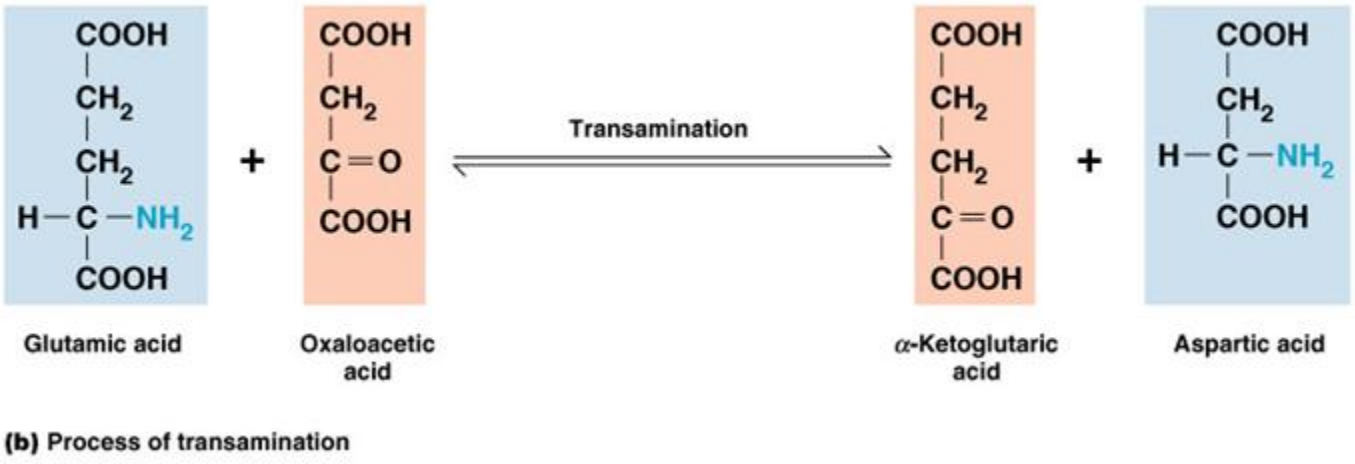
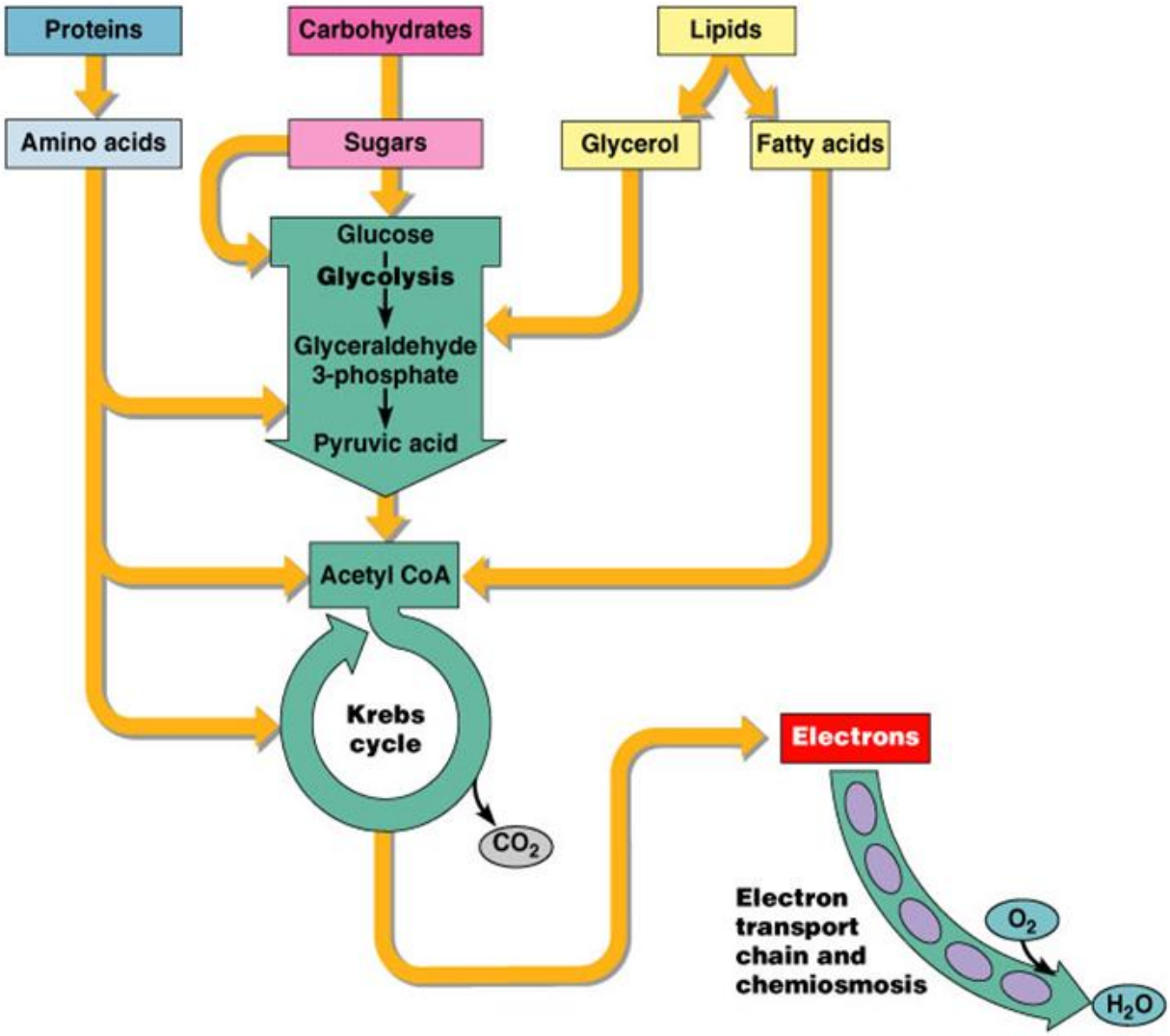


Fig.. Process of Transamination



Overview of catabolism of Organic Acids

Amino acid biosynthesis

Amino acid synthesis is the set of biochemical processes (metabolic pathways) by which the various amino acids are produced from other compounds. A fundamental problem for biological systems is to obtain nitrogen in an easily usable form. This problem is solved by certain microorganisms capable of reducing the inert N≡N molecule (nitrogen gas) to two molecules of ammonia in one of the most remarkable reactions in biochemistry. Ammonia is the source of nitrogen for all the amino acids. The carbon backbones come from the glycolytic pathway, the pentose phosphate pathway, or the citric acid cycle.

In amino acid production, one encounters an important problem in biosynthesis, namely stereochemical control. Because all amino acids except glycine are chiral, biosynthetic

pathways must generate the correct isomer with high fidelity. In each of the 19 pathways for the generation of chiral amino acids, the stereochemistry at the α-carbon atom is established by a transamination reaction that involves pyridoxal phosphate. Almost all the transaminases that catalyze these reactions descend from a common ancestor, illustrating once again that effective solutions to biochemical problems are retained throughout evolution.

Amino acid synthesis

Amino acids are synthesized from α-ketoacids and later transaminated from another aminoacid, usually Glutamate. The enzyme involved in this reaction is an aminotransferase.

α-ketoacid + glutamate ⇄ amino acid + α-ketoglutarate

Glutamate itself is formed by amination of α-ketoglutarate:

α-ketoglutarate + NH_{+4} ⇄ glutamate

Nitrogen fixation: Microorganisms use ATP and a powerful reductant to reduce atmospheric nitrogen to ammonia.

Microorganisms use ATP and reduced ferredoxin, a powerful reductant, to reduce N_2 to NH_3. An iron-molybdenum cluster in nitrogenase deftly catalyzes the fixation of N_2, a very inert molecule. Higher organisms consume the fixed nitrogen to synthesize amino acids, nucleotides, and other nitrogen-containing biomolecules. The major points of entry of NH^{4+} into metabolism are glutamine or glutamate.

- **Nitrifying bacteria**

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \xrightarrow{Nitrogenase} 2NH_3 + H_2 + 16ADP + 16P_i$$

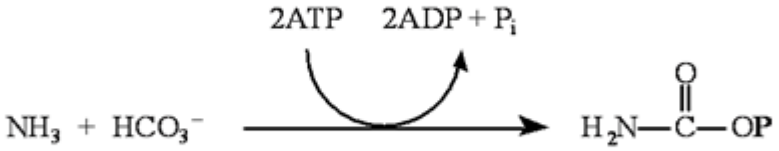
$$NH_3 \xrightarrow[\text{"Oxidation"}]{} NO_3^-$$

- **Nitrate Assimilation (Green plants, some fungi and bacteria)**

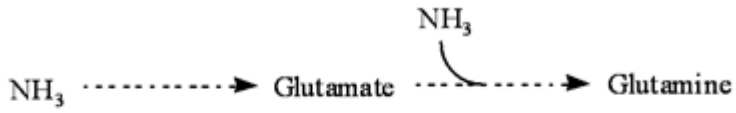
$$NO_3^- + NADH + H^+ \xrightarrow[\text{Nitrate Reductase}]{} NO_2^- + H_2O + NAD^+$$

$$NO_2^- + 8H^+ + 6e^- \xrightarrow[\text{Nitrite Reductase}]{} NH_4^+ + 2H_2O$$

- **Ammonium Assimilation (1) (Carbamyl Phosphate Synthetase)**



- **Ammonium Assimilation (2) (Biosynthetic Glutamate Dehydrogenase) and/or (Glutamine Synthetase)**



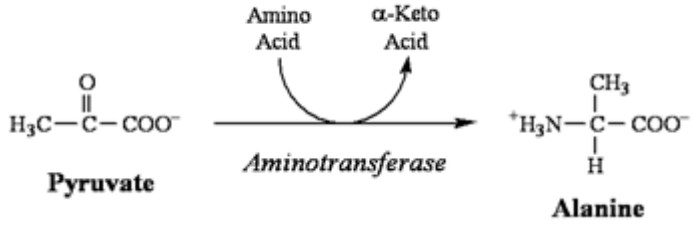
- **Glutamate (90%) and Glutamine (10%) are the main sources of organic Nitrogen for microbes.**

Glutamate (90%) ⟶ Amino Acids (90%)
Purines (50%)
Pyrimidines (50%)

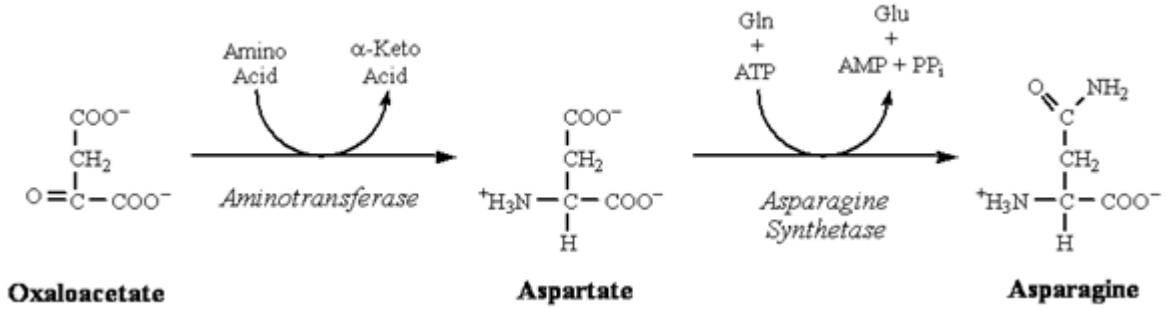
Glutamine (10%) ⟶ Amino Acids
Amino Sugars
NAD^+
PABA
Purines (50%)
Pyrimidines (50%)

Biosynthesis of some Non-essential Amino Acids (Reactions)

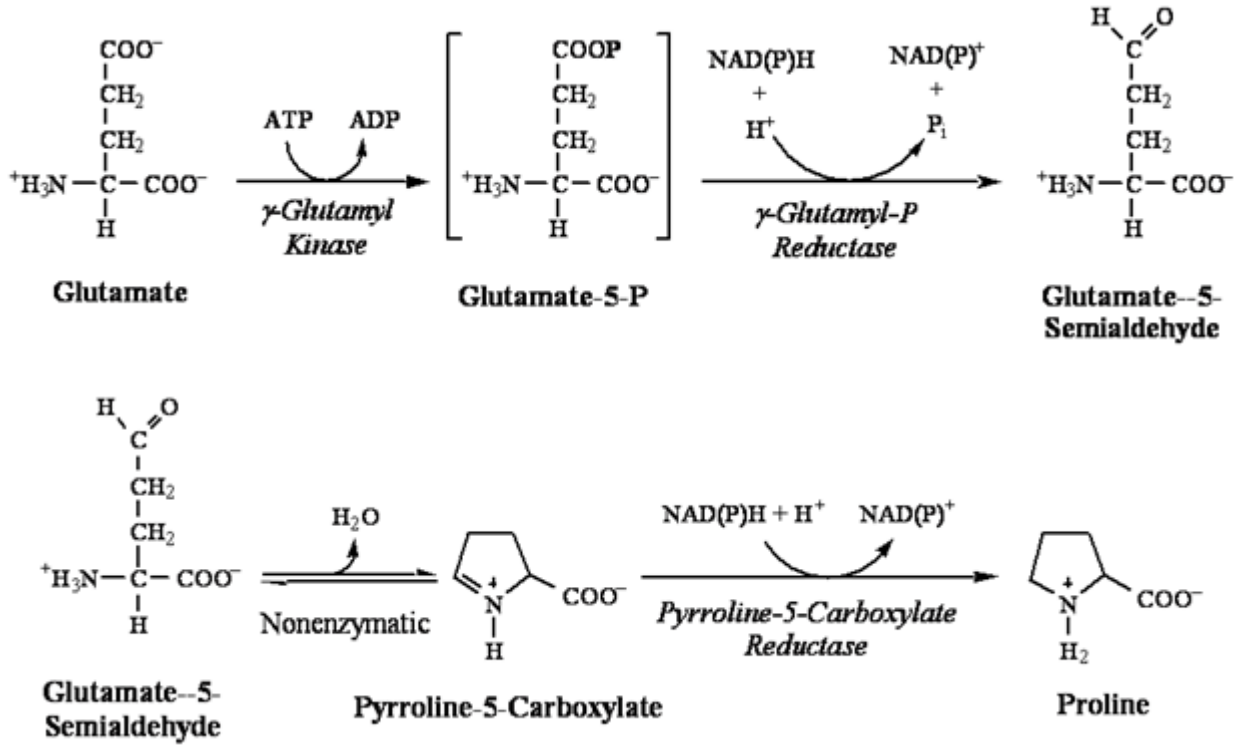
1. Alanine Biosynthesis



2. Aspartate and Asparagine Biosynthesis.



3. Proline Biosynthesis



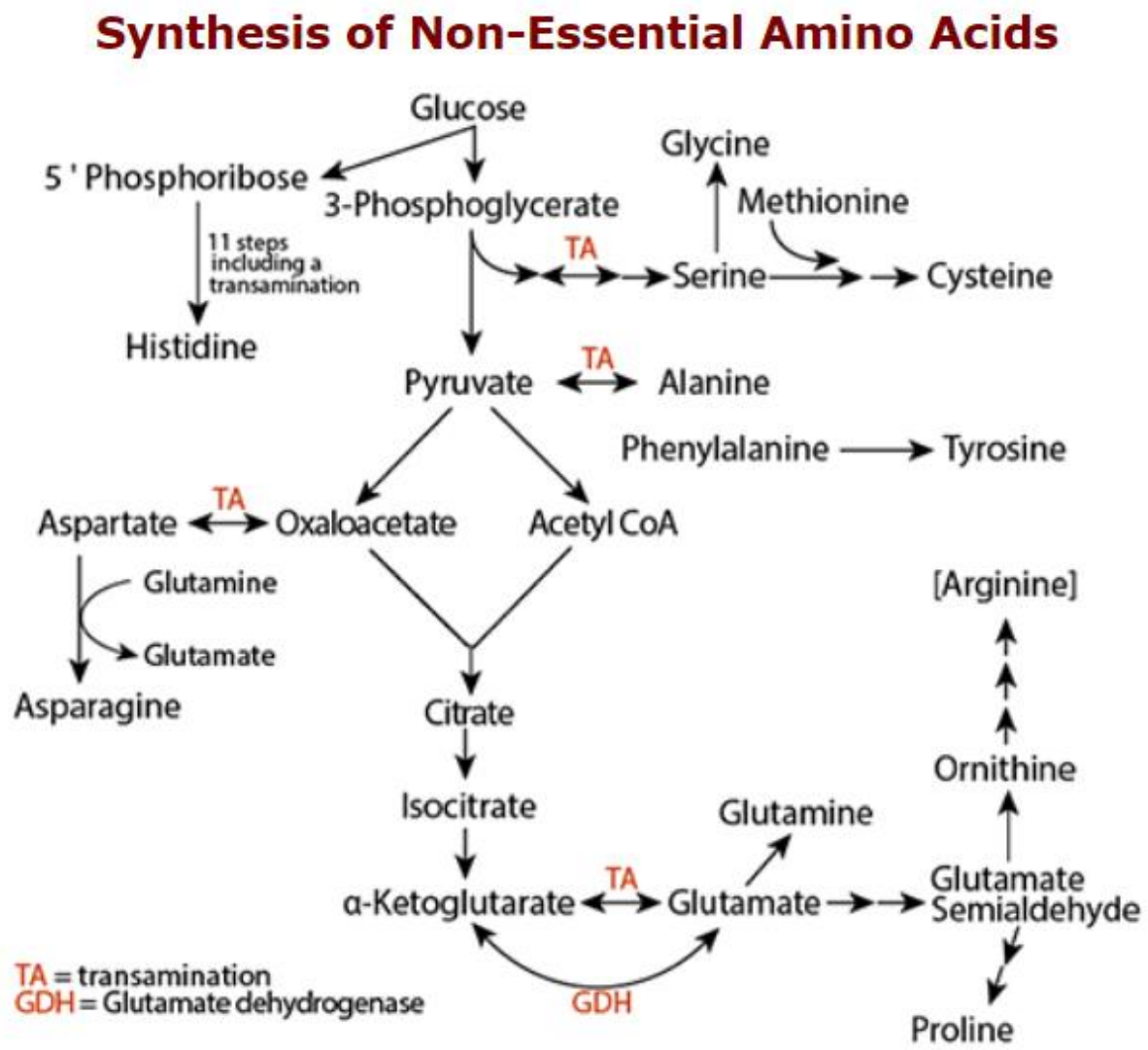
Amino acids are made from intermediates of the citric acid cycle and other major pathways

Glutamate dehydrogenase catalyzes the reductive amination of α-ketoglutarate to glutamate. A transamination reaction takes place in the synthesis of most amino acids. At this step, the chirality of the amino acid is established. Alanine and aspartate are synthesized by the transamination of pyruvate and oxaloacetate, respectively. Glutamine is synthesized from NH^{4+} and glutamate, and asparagine is synthesized similarly.

Proline and arginine are derived from glutamate. Serine, formed from 3-phosphoglycerate, is the precursor of glycine and cysteine. Tyrosine is synthesized by the hydroxylation of phenylalanine, an essential amino acid. The pathways for the biosynthesis of essential amino acids are much more complex than those for the nonessential ones. Activated Tetrahydrofolate, a carrier of one-carbon units, plays an important role in the metabolism of amino acids and nucleotides. This coenzyme carries one-carbon units at three oxidation states, which are interconvertible: most reduced—methyl; intermediate—methylene; and most oxidized—formyl, formimino, and methenyl.

Synthesis of the non-essential amino acids:

- Except for the synthesis of tyrosine from phenylalanine, carbon skeletons of the non-essential amino acids are produced from intermediates of glycolysis and the TCA cycle; four (serine, cysteine, glycine, alanine) from glycolytic intermediates, five (aspartate, asparagine, glutamic acid, glutamine, proline) from TCA cycle intermediates. Histidine is derived from glucose via the pentose phosphate pathway. Arginine is produced from ornithine by the urea cycle.
- Nitrogen is supplied as ammonia via transamination, using glutamic acid as the ammonia donor or, in the case of glutamic acid synthesis, by the reaction catalyzed by glutamate dehydrogenase.

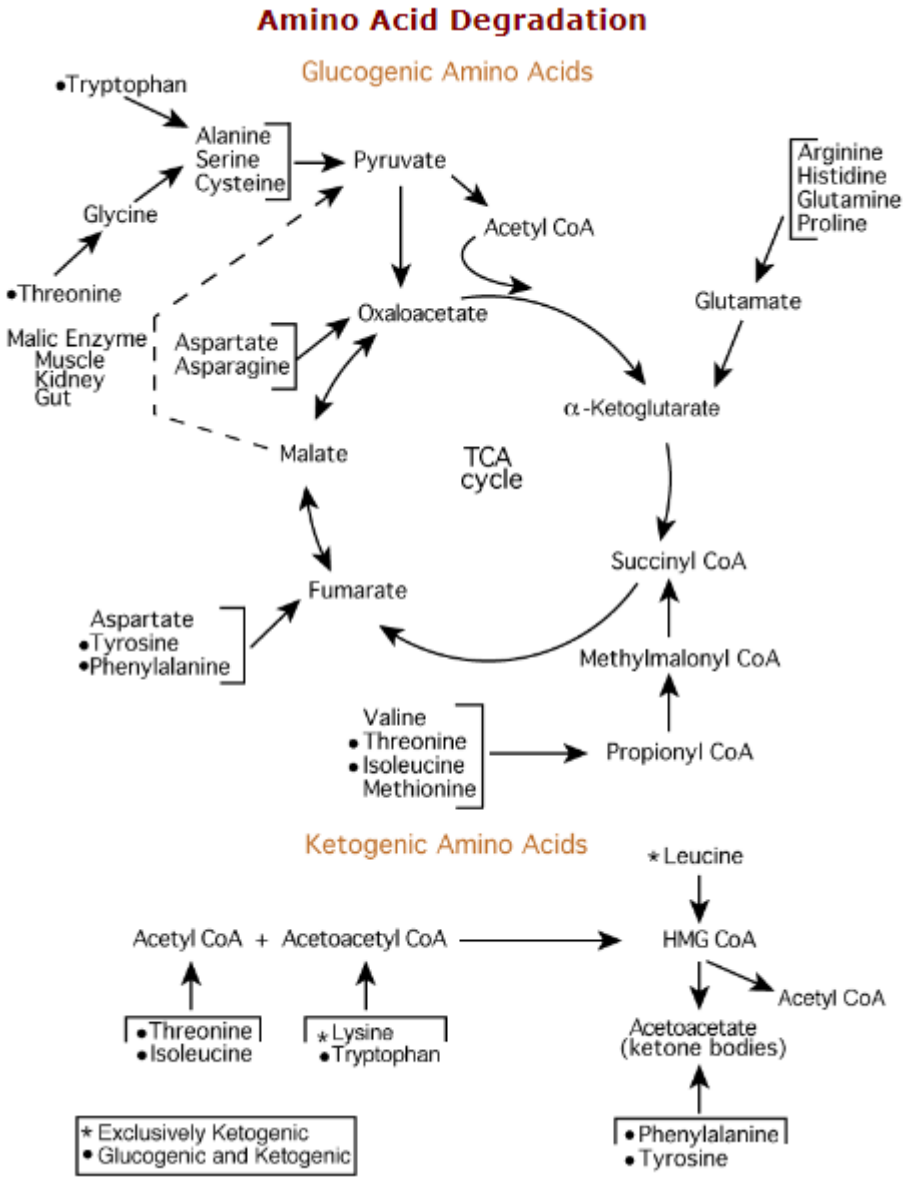


Amino acid degradation:

- Most amino acids are deaminated to produce α-keto acids. In the fed state these α-keto acids can be used to synthesize triacylglycerols. In the fasted state they produce glucose, ketone bodies and CO_2.
- In the fasted state, amino acids become a major source of energy. Muscle protein degradation supplies these amino acids, which the liver uses to synthesize the glucose and ketone bodies required to sustain life.
- Amino acids are considered to be glucogenic if their carbon skeletons can be converted, in net amounts, to glucose, and ketogenic if their carbon skeletons are converted directly to acetyl CoA or acetoacetate. Some amino acids are both glucogenic and ketogenic.
- 13 amino acids are exclusively glucogenic — alanine, arginine, aspartic acid,

asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, methionine, proline, serine, valine

- Two amino acids, leucine and lysine, are exclusively ketogenic
- Isoleucine, threonine and the aromatic amino acids — phenylalanine, tryptophan, tyrosine — are both glucogenic and ketogenic



Protein Synthesis:

During the 1950s and 1960s it became apparent that DNA is essential in the synthesis of proteins. Proteins are used as structural materials in the cells and function as enzymes. In addition, many specialized proteins function in cellular activities. For example, in bacteria, flagella and pili are composed of protein.

The genetic code. The key element of a protein molecule is how the amino acids are linked. The sequences of amino acids, determined by genetic codes in DNA, distinguish one protein from another. The **genetic code** consists of the sequence of nitrogenous bases in the DNA. How the nitrogenous base code is translated to an amino acid sequence in a protein is the basis for protein synthesis.

In order for protein synthesis to occur, several essential materials must be present. One is a supply of the 20 amino acids which make up most proteins. Another essential element is a series of enzymes that will function in the process. DNA and another form of nucleic acid called **ribonucleic acid (RNA)** are also essential. RNA carries instructions from the nuclear DNA into the cytoplasm, where protein is synthesized. RNA is similar to DNA, with three exceptions. First, the carbohydrate in RNA is ribose rather than deoxyribose. Second, RNA nucleotides contain the pyrimidine **uracil** rather than thymine. And third, RNA is usually single-stranded.

Types of RNA. In the synthesis of protein, three types of RNA are required. The first is called **ribosomal RNA (rRNA)** and is used to manufacture ribosomes. **Ribosomes** are ultramicroscopic particles of rRNA and protein where amino acids are linked to one another during the synthesis of proteins. Ribosomes may exist along the membranes of the endoplasmic reticulum in eukaryotic cells or free in the cytoplasm of prokaryotic cells.

A second important type of RNA is **transfer RNA (tRNA)**, which is used to carry amino acids to the ribosomes for protein synthesis. Molecules of tRNA exist free in the cytoplasm of cells. When protein synthesis is taking place, enzymes link tRNA to amino acids in a highly specific manner.

The third form of RNA is **messenger RNA (mRNA)**, which receives the genetic code from DNA and carries it into the cytoplasm where protein synthesis takes place. In this way, a genetic code in the DNA can be used to synthesize a protein at a distant location at the ribosome. The synthesis of mRNA, tRNA, and rRNA is accomplished by an enzyme called **RNA polymerase.**

Transcription. Transcription is one of the first processes in the overall process of protein synthesis. In transcription, a strand of mRNA is synthesized using the genetic code of DNA. RNA polymerase binds to an area of a DNA molecule in the double helix (the other strand remains unused). The enzyme moves along the DNA strand and selects complementary bases from available nucleotides and positions them in an mRNA molecule according to the principle of complementary base pairing (Figure 1). The chain of mRNA lengthens until a stop code is received.

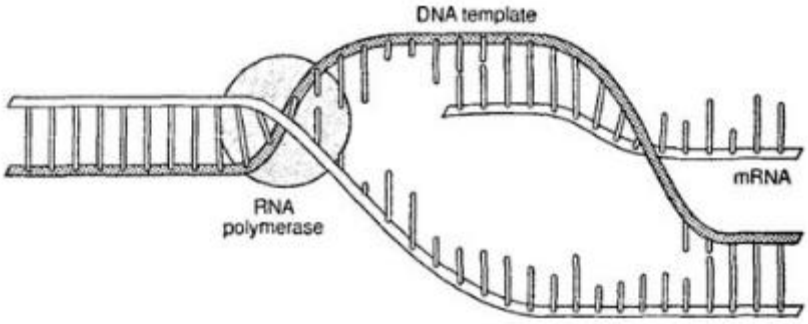


Figure 1

The synthesis of mRNA using a strand of DNA as a template.

The nucleotides of the DNA strands are read in groups of three. Each triplet is called a**codon**. Thus, a codon may be CGA, or TTA, or GCT, or any other combination of the four bases, depending on their sequence in the DNA strand. The mRNA molecule consists of a series of codons received from the genetic message in the DNA.

Once the stop codon has been reached, the mRNA molecule leaves the DNA molecule, and the DNA molecule rewinds to form a double helix. Meanwhile, the mRNA molecule proceeds thorough the cellular cytoplasm toward the ribosomes.

Translation. Translation is the process in which the genetic code will be "translated" to an amino acid sequence in a protein. The process begins with the arrival of the mRNA molecule at the ribosomes. While mRNA was being synthesized, tRNA molecules were uniting with their specific amino acids according to the activity of specific enzymes. The tRNA molecules then began transporting their amino acids to the ribosomes to meet the mRNA molecule.

After it arrives at the ribosomes, the mRNA molecule exposes its bases in sets of three, the codons. Each codon has a complementary codon called an **anticodon** on a tRNA molecule. When the codon of the mRNA molecule complements the anticodon on a tRNA molecule, the latter places the particular amino acid in that position. Then the next codon of the mRNA is exposed, and the complementary anticodon of a tRNA molecule matches with it. The amino acid carried by the second tRNA molecule is thus positioned next to the first amino acid, and the two are linked. At this point, the tRNA molecules release their amino acids and return to the cytoplasm to link up with new molecules of amino acid.

The ribosome then moves farther down the mRNA molecule and exposes another codon which attracts another tRNA molecule with its anticodon. Another amino acid is brought into position. In this way, amino acids continue to be added to the growing chain until the ribosome has moved down to the end of the mRNA molecule. The sequence of codons on the

mRNA molecule thus determines the sequence of amino acids in the protein being constructed (Figure 2).

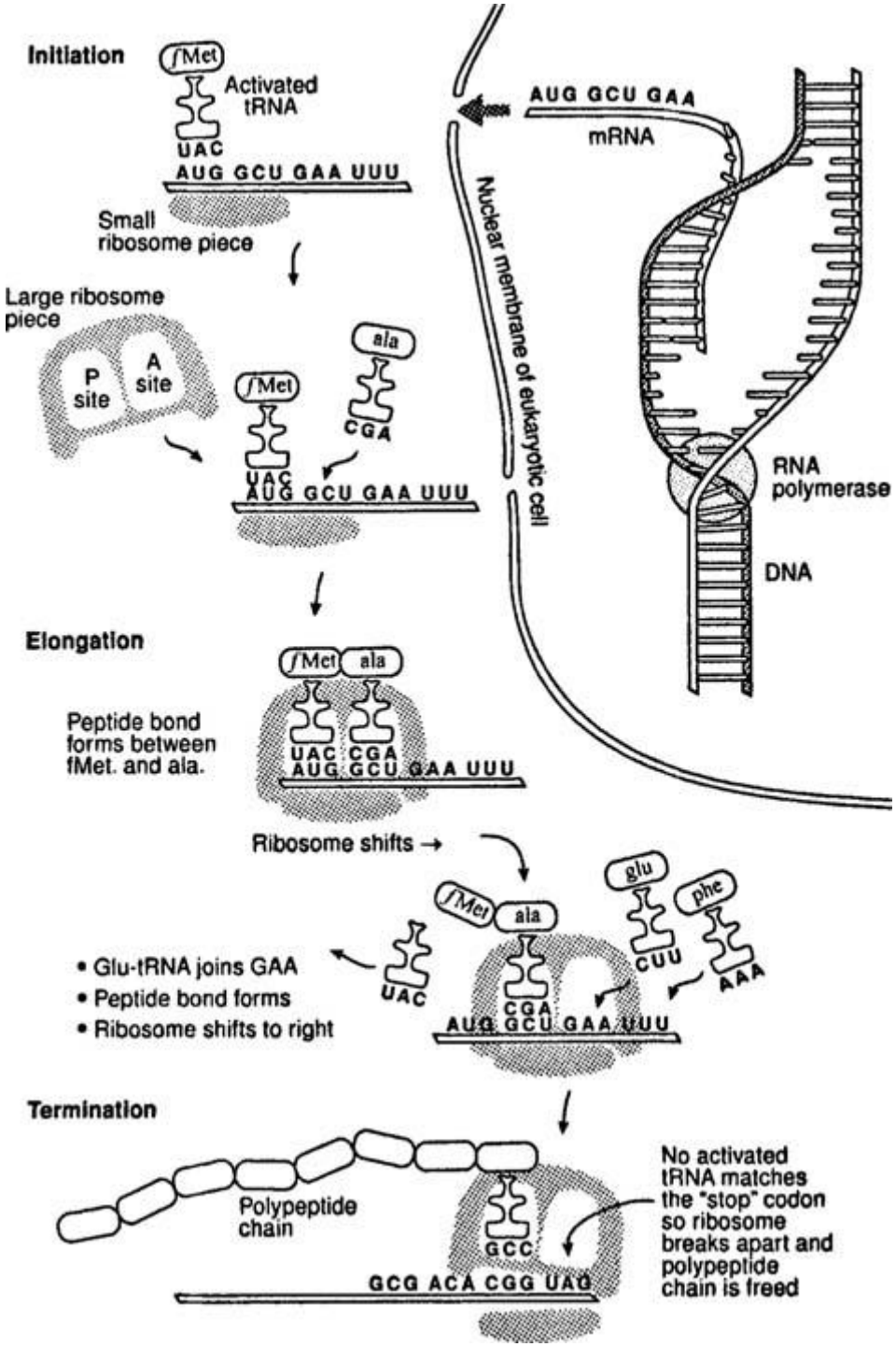


Figure 2

Steps in the synthesis of protein beginning with the genetic code in DNA and ending with the finished polypeptide chain.

Once the protein has been completely synthesized, it is removed from the ribosome for further processing. For example, the protein may be stored in the Golgi body of a eukaryotic cell before release, or a bacterium may release it as a toxin. The mRNA molecule is broken up and the nucleotides are returned to the nucleus. The tRNA molecules return to the cytoplasm to unite with fresh molecules of amino acids, and the ribosome awaits the arrival of a new mRNA molecule.

Gene control. The process of protein synthesis does not occur constantly in the cell, but rather at intervals followed by periods of genetic "silence." Thus, the process of gene expression is regulated and controlled by the cell.

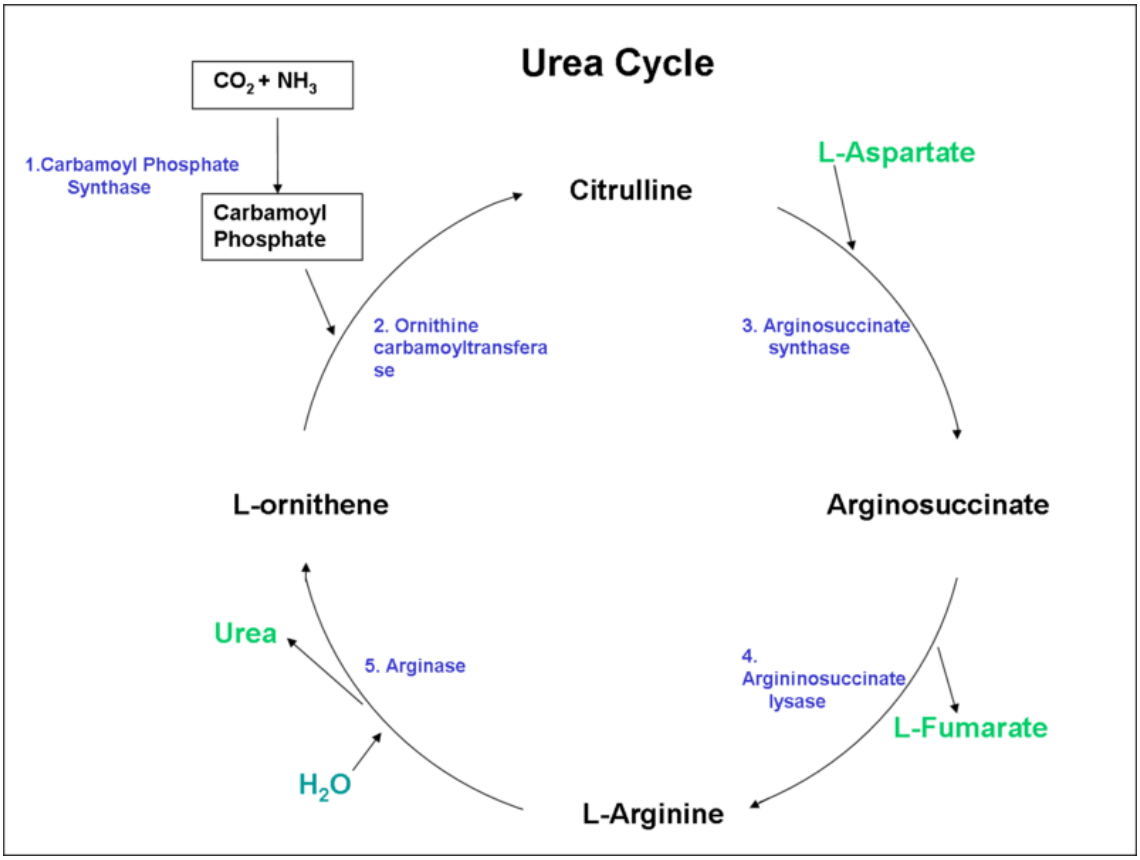
The control of gene expression can occur at several levels in the cell. For example, genes rarely operate during mitosis. Other levels of gene control can occur at transcription, when certain segments of DNA increase and accelerate the activity of nearby genes. After transcription has taken place, the mRNA molecule can be altered to regulate gene activity. For example, it has been found that eukaryotic mRNA contains many useless bits of RNA that are removed in the production of the final mRNA molecule. These useless bits of nucleic acid are called **introns.** The remaining pieces of mRNA, called **exons**, are then spliced to form the final mRNA molecule. Bacterial mRNA lacks introns.

The concept of gene control has been researched thoroughly in bacteria. In these microorganisms, genes have been identified as structural genes, regulator genes, and control regions. The three units form a functional unit called the **operon.**

The operon has been examined in close detail in certain bacteria. It has been found that certain carbohydrates can induce the presence of the enzymes needed to digest those carbohydrates. For example, when lactose is present, bacteria synthesize the enzymes needed to break it down. Lactose acts as an **inducer molecule** in the following way: In the absence of lactose, a regulator gene produces a repressor protein, which binds to a control region called the **operator site.** This binding prevents the structural genes from encoding the enzyme for lactose digestion. When lactose is present, however, it binds to the repressor protein and thereby removes the repressor at the operator site. With the operator site free, the structural genes are released to produce their lactose-digesting enzyme.

The urea cycle is the first metabolic pathway to be elucidated.

- The cycle is known as Krebs–Henseleit urea cycle.
- Ornithine is the first member of the reaction,
- It is also called as Ornithine cycle.
- The two nitrogen atoms of urea are derived from two different sources, one from ammonia & the other directly from the a- amino group of aspartic acid.
- Carbon atom is supplied by CO2
- Urea is the end product of protein metabolism (amino acid metabolism).
- Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol.



Steps of Urea Cycle:

1. **Formation of Carbamoyl Phosphate:**

Condensation of ammonium ion with bicarbonate ion resulting in the formation of carbamoyl phosphate by the help of the enzyme carbamoyl phosphate synthase-I present in the mitochondria. It requires Mg^{2+} and a dicarboxylic acid i.e. N-acetyl glutamate. This step requires 2 ATPs.

2. **Synthesis of Citrulline:**

Carbamoyl phosphate formed in the first step combines with ornithine resulting in the synthesis of citrulline aided by the enzyme citrulline synthase or ornithine transcarbamoylase. Citrulline is easily permeable to the mitochondrial membrane and hence it diffuses into the cytosol.

3. **Synthesis of Argininosuccinate:**

In the cytosol, citrulline combines with the amino acid aspartate forming argininosuccinate catalysed by the enzyme argininosuccinate synthase. It requires ATP which is hydrolysed to AMP resulting in utilization of two high energy bonds. Mg^{2+} acts as cofactor.

4. **Cleavage of Argininosuccinate:**

The enzyme argininosuccinase acts reversibly to cleave argininosuccinate into Arginine and fumarate. Fumarate enters the TCA cycle (the linkage between TCA and urea cycle is known as Krebs bi-cycle).

5. **Cleavage of Arginine:**

Arginine is lysed into ornithine and urea under the influence of the enzyme arginase. Hence arginine is known as semi-essential amino acid i.e. though it is synthesized in the body it is not available for protein synthesis. Ornithine is regenerated in this step and the urea cycle completes by the formation of urea. Ornithine and lysine are potent inhibitors of the enzyme arginase.

Arginase is also present in testis, renal tubules, mammary gland and skin in minute quantities. The intermediate amino acids formed in the urea cycle i.e. ornithine, citrulline and argininosuccinate are known as non-protein amino acids.

Anaerobic Respiration

Respiration in some prokaryotes is possible using electron acceptors other than oxygen (O_2). This type of respiration in the absence of oxygen is referred to as anaerobic respiration. Electron acceptors used by prokaryotes for respiration or methanogenesis (an analogous type of energy generation in archaea bacteria) are described in the table below.

Terminal e- Acceptor	Reduced End Product	Process	Example
O_2	H_2O	aerobic respiration	*Escherichia, Streptomyces*
NO_3	NO_2, NH_3 or N_2	anaerobic respiration: denitrification	*Bacillus, Pseudomonas*
SO_4	S or H_2S	anaerobic respiration: sulfate reduction	*Desulfovibrio*
fumarate	succinate	anaerobic respiration: using an organic e-acceptor	*Escherichia*
CO_2	CH_4	Methanogenesis	*Methanococcus*

Biological methanogenesis is the source of methane (natural gas) on the planet. Methane is preserved as a fossil fuel (until we use it all up) because it is produced and stored under anaerobic conditions, and oxygen is needed to oxidize the CH_4 molecule.

Methanogenesis is not really a form of anaerobic respiration, but it is a type of energy-generating metabolism that requires an outside electron acceptor in the form of CO_2.

Sulfate reduction is not an alternative to the use of O_2 as an electron acceptor. It is an obligatory process that occurs only under anaerobic conditions. Methanogens and sulfate reducers may share habitat, especially in the anaerobic sediments of eutrophic lakes such as Lake Mendota, where they crank out methane and hydrogen sulfide at a surprising rate.

Nitrate reduction

Some microbes are capable of using nitrate as their terminal electron accepter. The ETS used is somewhat similar to aerobic respiration, but the terminal electron transport protein donates its electrons to nitrate instead of oxygen. Nitrate reduction in some species (the best studied being *E. coli*) is a two electron transfer where nitrate is reduced to nitrite. Electrons flow through the quinone pool and the cytochrome b/c_1 complex and then nitrate reductase resulting in the transport of protons across the membrane as discussed earlier for aerobic respiration.

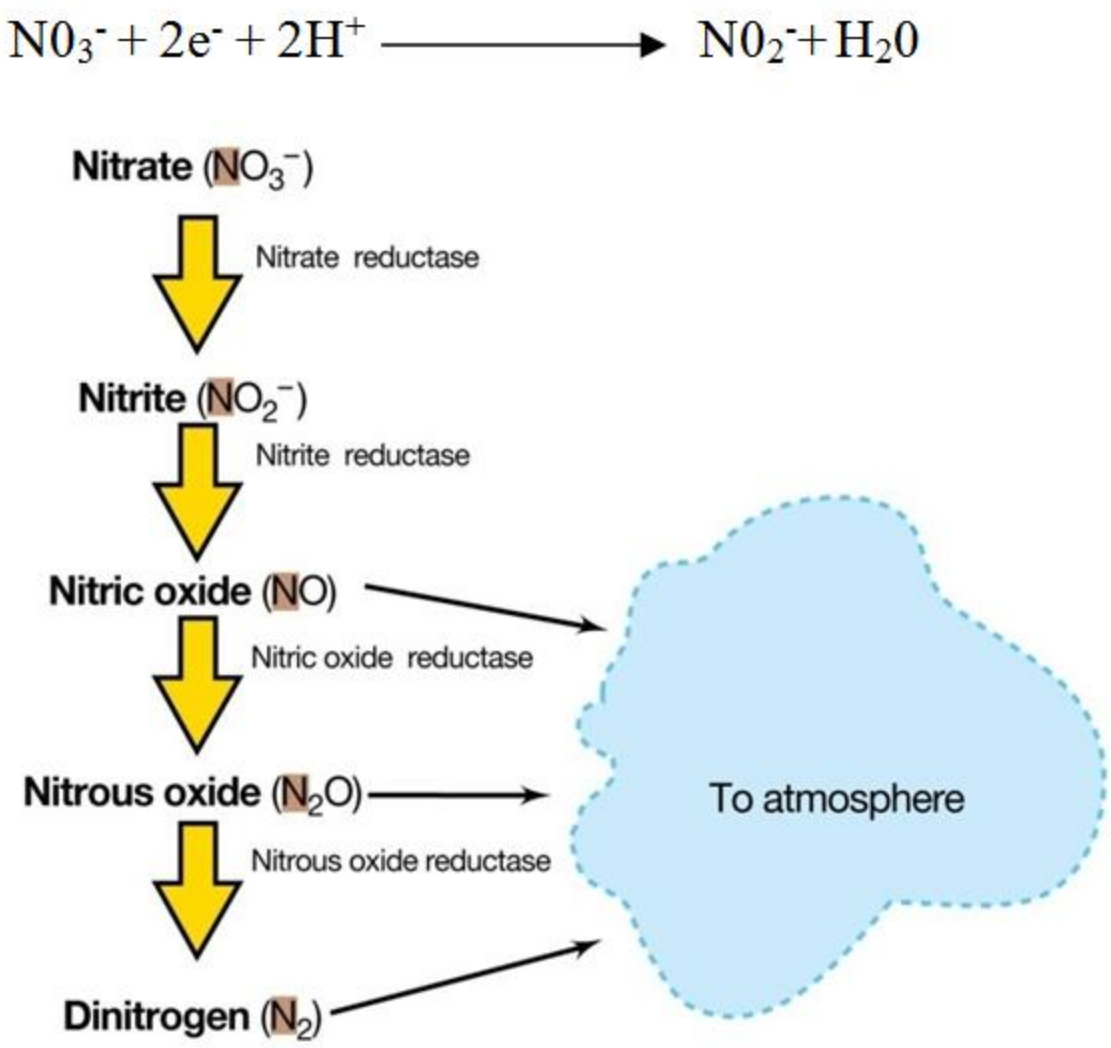


Fig. Nitrate reduction

Steps in the dissimilative reduction of nitrate . Some organisms, for example *Escherichia coli* , can carry out only the first step. All enzymes involved are derepressed by anoxic conditions. Also, some prokaryotes are known that can reduce NO_3^- to NH_4^+ in dissimilative metabolism.

Denitrification

Denitrification is an important process in agriculture because it removes NO_3 from the soil. NO_3 is a major source of nitrogen fertilizer in agriculture. Almost one-third the cost of some types of agriculture is in nitrate fertilizers. The use of nitrate as a respiratory electron acceptor is usually an alternative to the use of oxygen. Therefore, soil bacteria such as *Pseudomonas* and *Bacillus* will use O_2 as an electron acceptor if it is available, and disregard NO_3. This is the rationale in maintaining well-aerated soils by the agricultural

practices of plowing and tilling. *E. coli* will utilize NO_3 (as well as fumarate) as a respiratory electron acceptor and so it may be able to continue to respire in the anaerobic intestinal habitat.

Nitrite, the product of nitrate reduction, is still a highly oxidized molecule and can accept up to six more electrons before being fully reduced to nitrogen gas. Microbes exist (*Paracoccus species*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa* , and *Rhodobacter sphaeroides* are a few examples) that are able to reduce nitrate all the way to nitrogen gas. The process is carefully regulated by the microbe since some of the products of the reduction of nitrate to nitrogen gas are toxic to metabolism. This may explain the large number of genes involved in the process and the limited number of bacteria that are capable of denitrification. Below is the chemical equation for the reduction of nitrate to N_2.

$$N0_3^- \longrightarrow N0_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

Denitrification takes eight electrons from metabolism and adds them to nitrate to form N_2

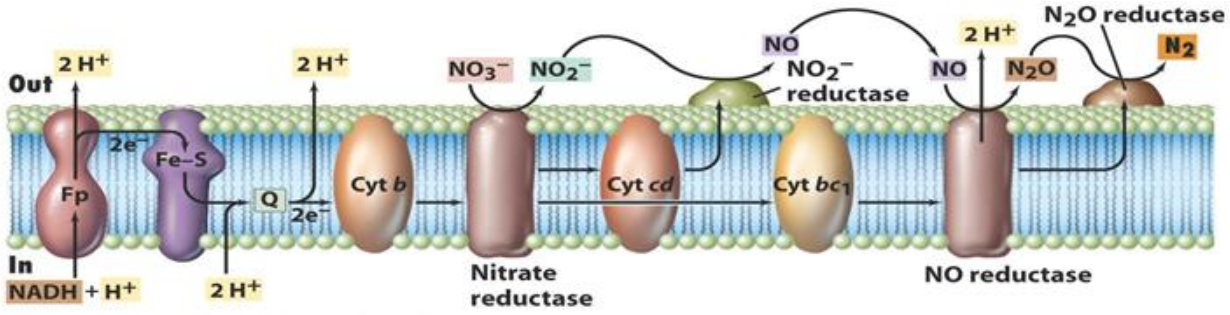


Fig. Denitification by *Pseudomonas stutzeri*

- Four terminal reductases involved in denitrification steps;

- **Nar** : Nitrate reductase (Mo-containing enzyme)

- **Nir** : Nitrite reductase

- **Nor** : Nitric oxide reductase

- **N_2 Or** : Nitrous oxide reductase

- All can function independently but they operate in unison

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. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis.

During fermentation, **pyruvate is metabolised to various compounds**. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols.

Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (a phenomenon known as the **Crabtree effect**).

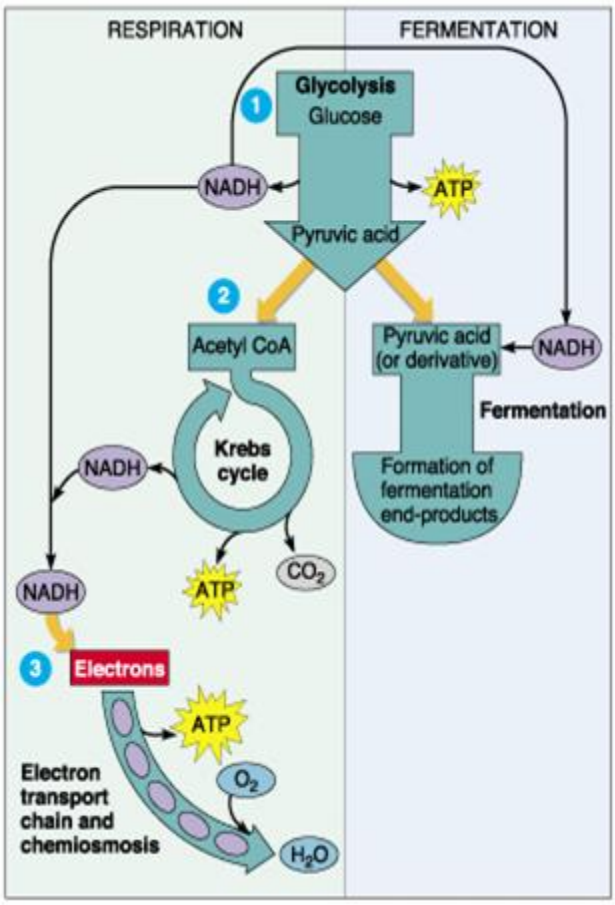


Fig. Respiration and Fermentation pathways

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces – transfers electrons to – NAD+, forming NADH. However there is a limited supply of NAD+ available in any given cell.

- • For glycolysis to continue, NADH must be oxidized – have electrons taken away – to regenerate the NAD+ that is used in glycolysis. In an aerobic environment (Oxygen is available), reduction of NADH is usually done through an electron transport chain in a process called oxidative phosphorylation; however, oxidative phosphorylation cannot occur in anaerobic environments (Oxygen is not available) due to the pathways dependence on the terminal electron acceptor of oxygen.

  • Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate.

  In heterolactic acid fermentation, one molecule of pyruvate is converted to lactate; the other is converted to ethanol and carbon dioxide.

  In homolactic acid fermentation, both molecules of pyruvate are converted to lactate. Homolactic acid fermentation is unique because it is one of the only respiration processes to not produce a gas as a byproduct.

- • Homolactic fermentation breaks down the pyruvate into lactate. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen.

  • It also occurs in some kinds of bacteria (such as **lactobacilli**) and some fungi. It is this type of bacteria that converts lactose into lactic acid in yogurt, giving it its sour taste. These lactic acid bacteria can be classed as homofermentative, where the end-product is mostly lactate, or heterofermentative, where some lactate is further metabolized and results in carbon dioxide, acetate, or other metabolic products.

  $C_6H_{12}O_6$ ------→ $2CH_3CHOHCOOH$.

  or one molecule of lactose and one molecule of water make four molecules of lactate (as in some yogurts and cheeses):

  $C_{12}H_{22}O_{11}+H_2O$ -----→ $4CH_3CHOHCOOH$.

In heterolactic fermentation, the reaction proceeds as follows, with one molecule of glucose being converted to one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide:

$C_6H_{12}O_6$ --------→ $CH_3CHOHCOOH + C_2H_5OH + CO_2$

Before lactic acid fermentation can occur, the molecule of glucose must be split into two molecules of pyruvate. This process is called glycolysis.

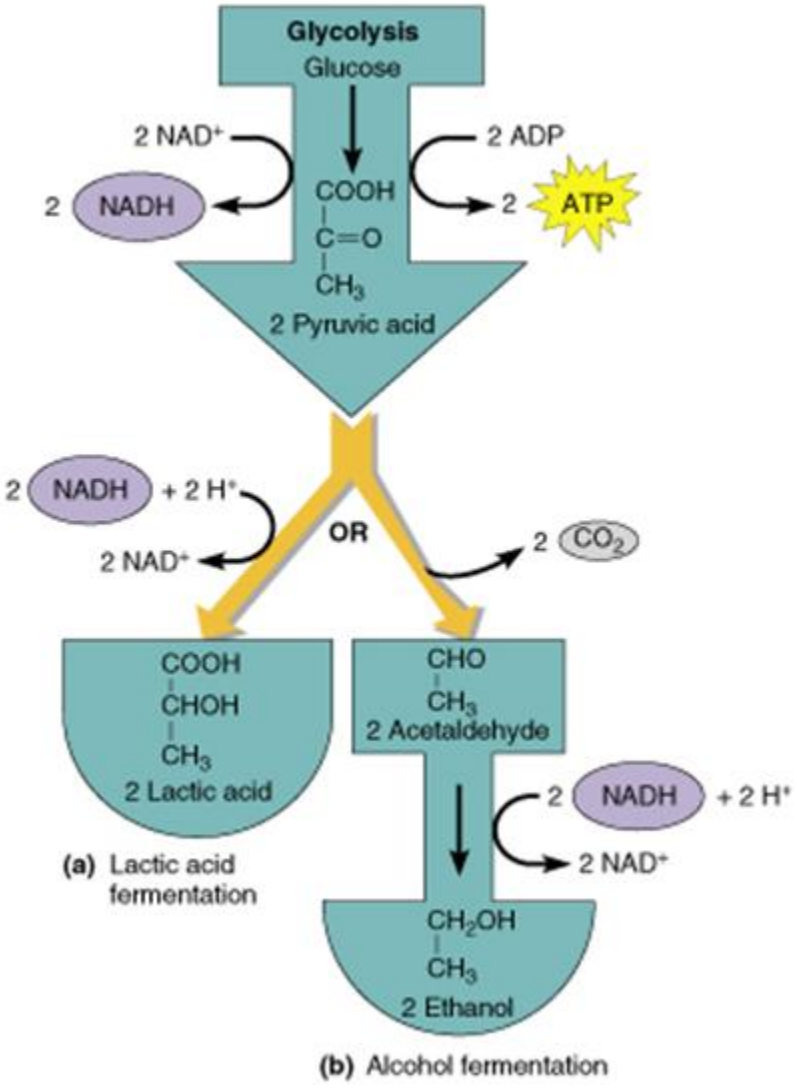


Fig. Fate of pyruvate in Fermentation

Mixed fermentations

Butanediol Fermentation. Forms mixed acids and gases as above, but, in addition, **2,3 butanediol** from the condensation of 2 pyruvate. The use of the pathway decreases acid formation (butanediol is neutral) and causes the formation of a distinctive

intermediate, **acetoin**. Water microbiologists have specific tests to detect low acid and acetoin in order to distinguish non fecal enteric bacteria (butanediol formers, such as *Klebsiella* and *Enterobacter*) from fecal enterics (mixed acid fermenters, such as *E. coli, Salmonella* and *Shigella*).

Butyric acid fermentations, as well as the butanol-acetone fermentation (below), are run by the clostridia, the masters of fermentation. In addition to butyric acid, the clostridia form acetic acid, CO_2 and H_2 from the fermentation of sugars. Small amounts of ethanol and isopropanol may also be formed.

Butanol-acetone fermentation. Butanol and acetone were discovered as the main end products of fermentation by *Clostridium acetobutylicum* during the World War I. This discovery solved a critical problem of explosives manufacture (acetone is required in the manufacture gunpowder) and is said to have affected the outcome of the War. Acetone was distilled from the fermentation liquor of *Clostridium acetobutylicum*, which worked out pretty good if you were on our side, because organic chemists hadn't figured out how to synthesize it chemically. You can't run a war without gunpowder, at least you couldn't in those days.

Propionic acid fermentation . This is an unusual fermentation carried out by the propionic acid bacteria which include corynebacteria, *Propionibacterium* and *Bifidobacterium* . Although sugars can be fermented straight through to propionate, propionic acid bacteria will ferment lactate (the end product of lactic acid fermentation) to acetic acid, CO_2 and propionic acid. The formation of propionate is a complex and indirect process involving 5 or 6 reactions. Overall, 3 moles of lactate are converted to 2 moles of propionate + 1 mole of acetate + 1 mole of CO_2, and 1 mole of ATP is squeezed out in the process. The propionic acid bacteria are used in the manufacture of Swiss cheese, which is distinguished by the distinct flavor of propionate and acetate, and holes caused by entrapment of CO_2.

Lipid Catabolism

Microorganisms frequently use lipids as energy sources. Triglycerides or triacylglycerols, esters of glycerol and fatty acids, are common energy sources. They can be hydrolyzed to glycerol and fatty acids by microbial lipases. The glycerol is then phosphorylated, oxidized to dihyroxyacetone phosphate, and catabolised in the glycolytic pathway.

1. Lipases are enzymes that break down fats into fatty acid and glycerol components

2. Beta oxidation is the breakdown of fatty acids into two carbon segments (acetyl CoA),

Which can enter the Krebs cycle.

Functions of lipids in Microbes

• Lipids are essential to the structure and function of membranes

• Lipids also function as energy reserves, which can be mobilized as sources of carbon

• 90% of this lipid is “triacyglycerol”

Triacyglycerol **-----lipase----->** glycerol + 3 fatty acids

• The major fatty acid metabolism is “β-oxidation”

Lipids are broken down into their constituents of glycerol and fatty acids

Glycerol is oxidised by glycolysis and the TCA cycle.

• Bacteria are capable of growth on fatty acids and lipids. Lipids are part of the membranes of living organisms and if available (usually because the organism that was using them dies) can be used as a food source.

• Lipids are large molecules and cannot be transported across the membrane.

• A class of extracellular enzymes called **lipases** are responsible for the breakdown of lipids. Lipases attack the bond between the glycerol molecule oxygen and the fatty acid.

• Phospholipids are attacked by **phospholipases.** There are four classes of phospholipases given different names depending upon the bond they cleave. Phospholipases are not particular about their substrate and will attack a glycerol ester linkage containing any length fatty acid attached to it. The result of this digestion is a hydrophillic head molecule, glycerol and fatty acids of various chain lengths.

• The head can be one of several small organic molecules that are funneled into the TCA cycle by one or two reactions that we won't cover here.

• Glycerol is converted into 3-Phosphoglycerate (depending upon the action of phospholipase C or phospholipase D) and eventually pyruvate via glycolysis.

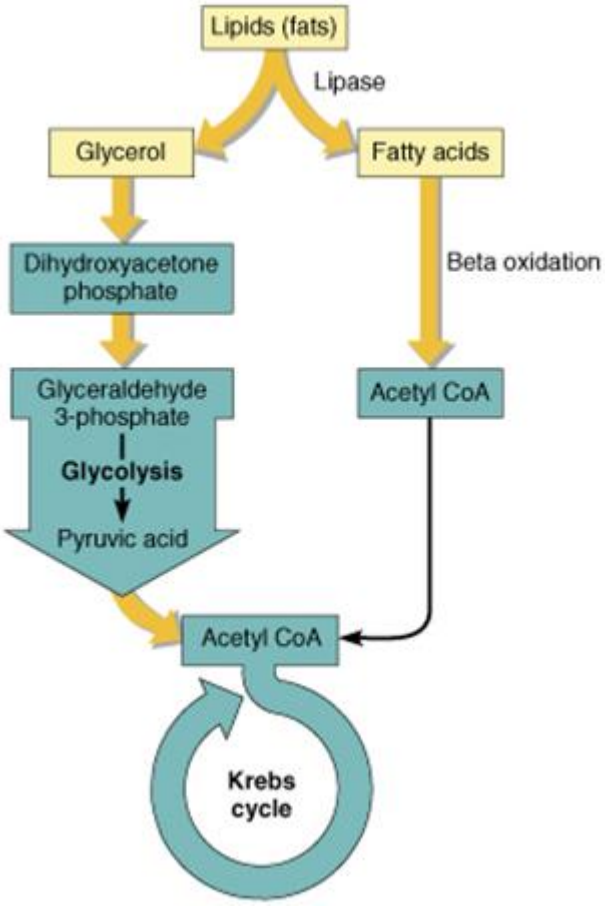


Fig. Lipid Catabolism

The β- oxidation pathway

Characteristic features;

- Every other carbon is converted to a C=O
- Allows nucleophilic attack by CoA-SH on remaining chain
- 1 CoA is used for every 2 carbon segment to release acetyl-CoA
- Each round produces

1 $FADH_2$, 1 NADH, 1 Acetyl-CoA (2 in the last round)

Step 1: Dehydrogenation of Alkane to Alkene Catalyzed by isoforms of acyl-CoA dehydrogenase (AD) on the inner mitochondrial membrane

Step 2: Hydration of Alkene Catalyzed by two isoforms of enoyl-CoA hydratase:

Soluble short-chain hydratase (crotonase) Membrane-bound long-chain hydratase, part of trifunctional complex Water adds across the double bond yielding alcohol

Step 3: Dehydrogenation of Alcohol Catalyzed by β-hydroxyacyl-CoA dehydrogenase The enzyme uses NAD cofactor as the hydride acceptor Only L-isomers of hydroxyacyl CoA act as substrates Analogous to malate dehydrogenase reaction in the CAC.

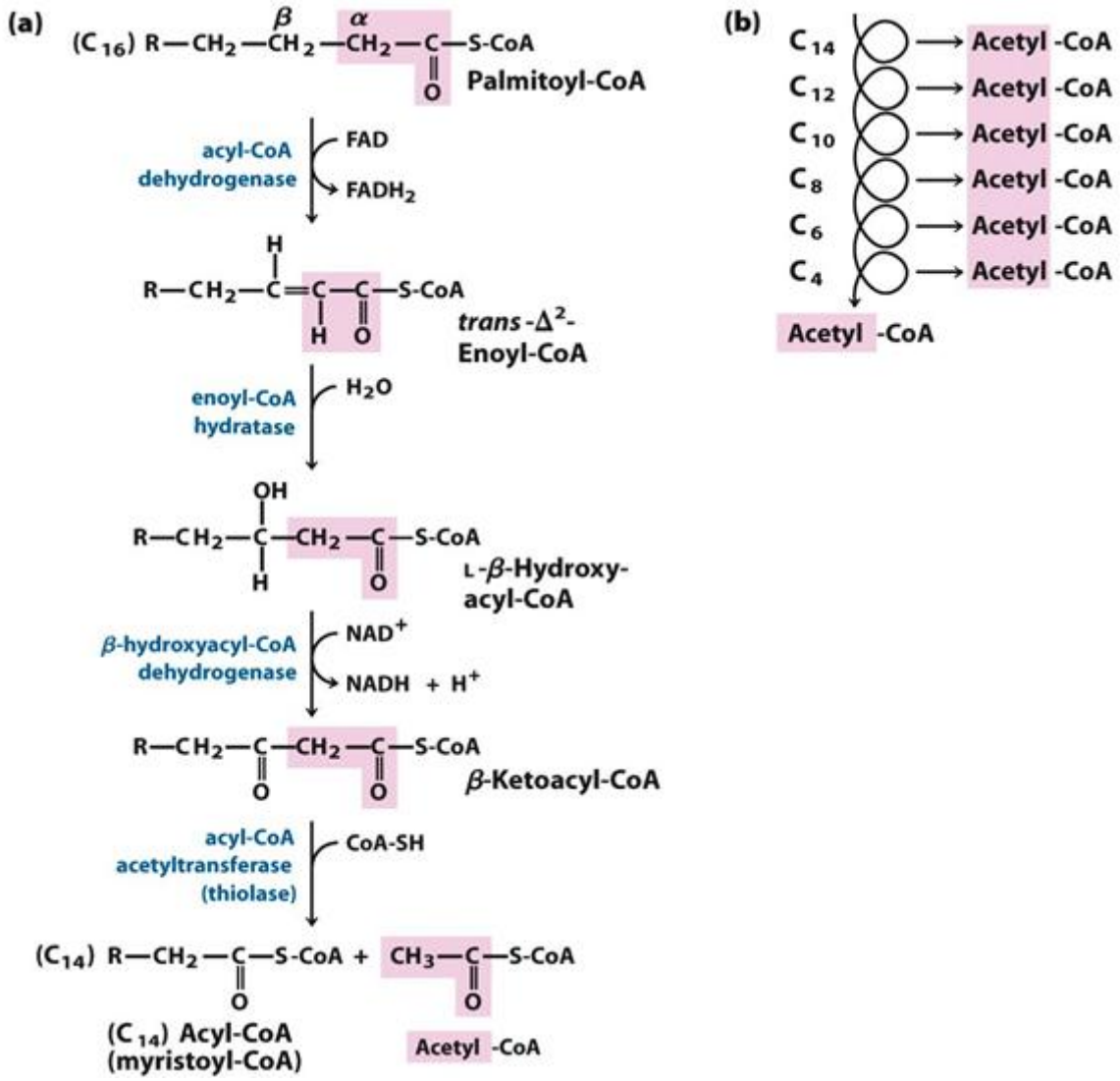


Fig. The β- oxidation pathway

Step 4: Transfer of Fatty Acid Chain Catalyzed by acyl-CoA acetyltransferase (thiolase) via covalent mechanism, The carbonyl carbon in b -ketoacyl-CoA is electrophilic Active site thiolate acts as nucleophile and releases acetyl-CoA; Terminal sulfur in CoA-SH acts as nucleophile.

The fatty acid is now two carbons shorter and an **Acetyl-CoA,** has been generated which can be fed into the TCA cycle. The smaller fatty acid moves through the β-oxidation pathway again, producing another Acetyl-CoA and shrinking by 2 carbons.

By performing successive rounds of beta oxidation on a fatty acid, it is possible to convert it completely to Acetyl-CoA. Often fatty acids with odd numbers of carbons, the final reaction will yield acetyl-CoA and Coenzyme-A hooked to a three carbon fatty acid (propionyl-CoA). Propionyl-CoA is handled differently by different bacteria. In *E. coli* it is converted into pyruvate.