UNIT-IV CHEMOLITHOTROPHIC AND PHOTOTROPHIC METABOLISM

CHEMOLITHOTROPHS:

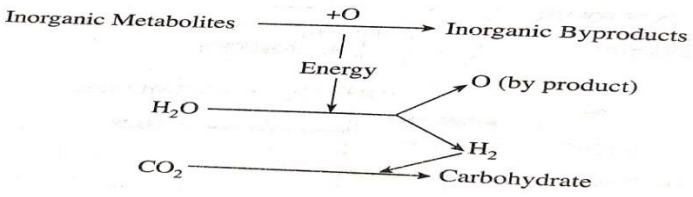
Microbes that synthesize ATP with the energy liberated when they oxidize organic substrates such as carbohydrates, lipids, and proteins. The electron acceptor is: (1) O_2 in aerobic respiration, (2) an oxidized exogenous molecule other than O_2 in anaerobic respiration, or (3) another more oxidized endogenous organic molecule (usually pyruvate) in fermentation. In fermentation, ATP is synthesized only by substrate-level phosphorylation; in both aerobic and anaerobic respiration, most of the ATP is formed using the PMF derived from electron transport chain activity. Additional metabolic diversity among bacteria and archaea is reflected in the form of energy metabolism performed by chemolithotrophs. These microbes obtain electrons for the electron transport chain from the oxidation of inorganic molecules rather than NADH generated by the oxidation of organic nutrients Each species is rather specific in its preferences for electron donors and acceptors. The acceptor is usually O_2, but sulfate and nitrate are also used. Themost common electron donors are hydrogen ,reduced nitrogen compounds, reduced sulfur compounds, and ferrous iron (Fe2). Much less energy is available from the oxidation of inorganic molecules than from the complete oxidation of glucose to CO2, which is accompanied with a standard free energy change of 686 kcal/mole. This is because the NADH that donates electrons to the chain following the oxidation of an organic substrate like glucose has a more negative reduction potential than most of the inorganic substrates that chemolithotrophs use as direct electron donors to their electron transport chains. Thus the P/O ratios for oxidative phosphorylation in chemolithotrophs are probably around 1.0 (although in the oxidation of hydrogen it is considerably higher). Because the yield ofATPis so low, chemolithotrophs must oxidize a large quantity of inorganic material to grow and reproduce. This is particularly true of autotrophic chemolithotrophs, which fix CO_2 intocarbohydrates .For each molecule of CO_2 fixed, these microbes expendthree ATP and two NADPH molecules. Because they must consume a large amount of inorganic material, chemolithotrophs have significant ecological impact.

Representative Chemolithotrophs and Their Energy Sources

Bacteria	Electron Donor	Electron Acceptor	Products
Alcaligenes, Hydrogenophaga, and *Pseudomonas* spp.	H_2	O_2	H_2O
Nitrobacter	NO_2^-	O_2	NO_3^-, H_2O
Nitrosomonas	NH_4^+	O_2	NO_2^-, H_2O
Thiobacillus denitrificans	S^0, H_2S	NO_3^-	SO_4^{2-}, N_2
Thiobacillus ferrooxidans	Fe^{2+}, S^0, H_2S	O_2	Fe^{3+}, H_2O, H_2SO_4

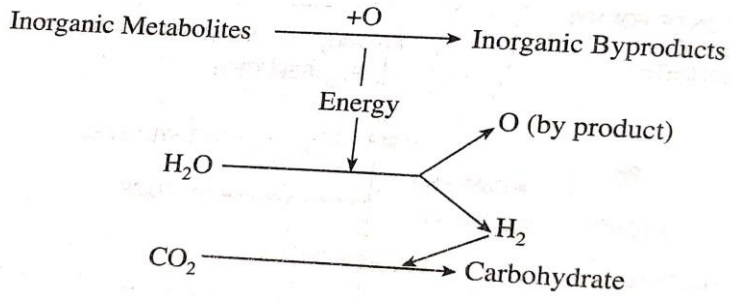
This is a group of non-photosynthetic autotrophic microorganisms consisting entirely of bacteria. They cannot use light, and their external energy sources in food manufacture are a variety of inorganic metabolites absorbed from the environment. In the most cases, these (exothermic reaction) and a variety of inorganic byproducts. Water and carbon dioxide are the inorganic raw materials in subsequent food manufacture. The concept of chromoautotrophy (chemolithotrophy) was formulated by Winogradsky. By studying Beggiatoa, he demonstrated

for the first time that a living organism could oxidize H_2S to elemental sulphur and then to so. This process of manufacturing food is called chemosynthesis. The general pattern is as follows.



Among the best known chemoautotrophic microorganisms are the sulphur-oxidizing bacteria, the iron-oxidizing bacteria, the nitrifying bacteria, and the hydrogen-oxidizing bacteria

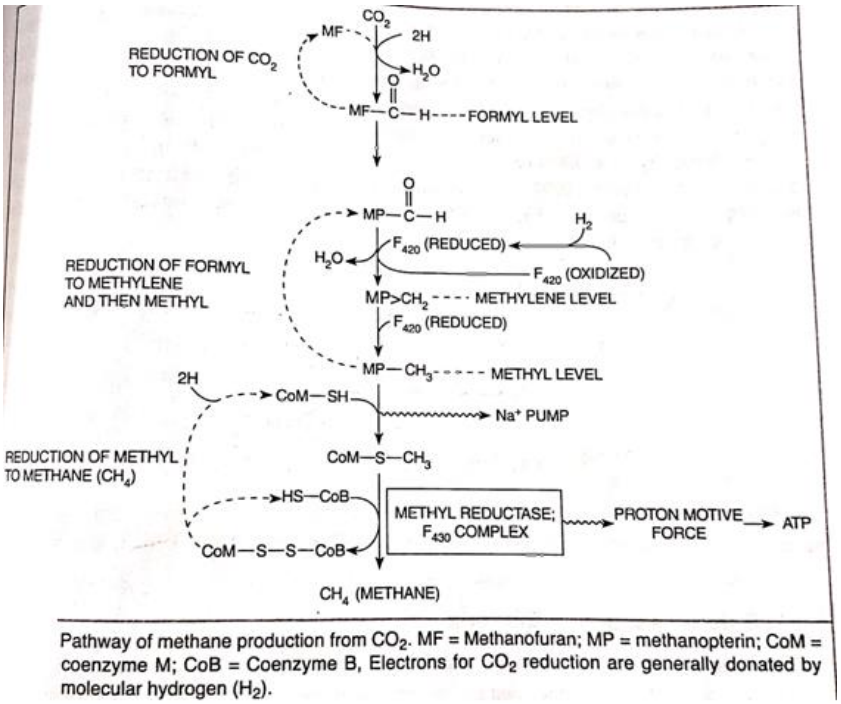
Hydrogen-oxdising Bacteria: Hydrogen-oxidising bacteria represent the group of bacteria that oxidise H, (the sole electron donor) and reduce O_2 (the electron acceptor) via "knallgas" reaction, the reduction of O_2 with H_2. This reaction yields energy (-273 kJ/reaction), which is used in CO_2 fixation. These bacteria are both gram-positive and gram-negative. The best studied genera of this group of bacteria are *Ralstonia, Pseudomonas, Paracoccus, and Alkaligenes; others are Acidovorax, Aquaspirillum, Hydrogenophaga, Hydrogenobacter, Bacillus, Aquifex, and Mycobacterium.* All hydrogen-oxidising bacteria possess one or more hydrogenase enzymes that function to bind hydrogen (H_2) and use it either to generate ATP or for reducing power for chemoautotrophic growth. Most hydrogen-oxidising bacteria flourish best under microaerobic conditions when growing chemoautotrophically (chemolithotrophically) on hydrogen because hydrogenases are oxygen-sensitive enzymes. Typically, oxygen levels of about 5-10% support best growth of these bacteria. The general pattern of the nutritional life-style of chemoautotrophic (chemolithotrophic) hydrogen-oxidizing bacteria is as follows :



Almost all hydrogen-oxidising bacteria are facultative chemoautotrophs, i.e, they can also grow chemoheterotrophically (chemoorganotrophically) with organic compounds as energy source. This means that the hydrogen-oxidising bacteria have ability to switch between chemoautotrophic and chemoheterotrophic (chemoorganotrophic) modes of metabolism and generally do so in nature whenever required. This is a major distinction between hydrogenoxidising bacteria and many sulphur-oxidising bacteria or nitrifying bacteria; most of

the from vFlatlatter two groups are obligate chemoautotrophs (i.e., their growth fails to occur in the absence of the inorganic energy source). from vFlat

2. Methanogenesis: Methanogenesis (methane production) is characteristic to a group of obligate anaerobic archaea (archaebacteria) called the methanogens (e.g., *Methanobacterium, Methanobrevibacter, Methanococcus, Methanogenium, Methanospirillum, Methanomicrobium,* etc.). The reduction of CO_2 to CH_4 by methanogens is generally dependent on H_2 that donates electrons. However, formate, CO_2 and even certain organic compounds such as alcohol may also be used as electron donors in the process. Studies on methanogenesis have revealed that the biological production of methane takes place through a series of reactions involving novel coenzymes and amazing complexity. However, the reduction of CO_2 to methane can be summarised in the following way



Pathway of methane production from CO_2. MF = Methanofuran; MP = methanopterin; CoM = coenzyme M; CoB = Coenzyme B, Electrons for CO_2 reduction are generally donated by molecular hydrogen (H_2).

CO_2 is activated by a methanofuran (MF)-containing enzyme and is reduced to formyl level. The formyl group is transferred from methanofuran to an enzyme bonded with methanopterin (MP) and is subsequently dehydrated and reduced to methylene and methyl levels. The methyl group is transferred from methanopterin to'an enzyme containing coenzyme M (COM). Methyl-CoM is reduced to methane by the methyl reductase system in F_{430} and coenzyme B (CoB) are intimately involved. Coenzyme F_{430} removes the CH_4 group from COM-S-CH_3. The CH_4 group is reduced by electrons from coenzyme B (COB) generating methane (CHA) and a disulphide complex of CoM and CoB (COM-S-S-CoB). Free CoM and CoB are regenerated by reduction of this complex with molecular hydrogen (H_2). It is this reaction during which the energy is conserved in the form of ATP with the involvement of proton motive force.

PHOTOTROPHIC METABOLISM

PHOTOTROPHY

Microorganisms derive energy not only from the oxidation of inorganic and organic compounds, but also from light energy, which they capture and use to synthesize ATP and reduce power (e.g., NADPH). The process by which light energy is trapped and converted to chemical energy is called photosynthesis. Usually a phototrophic organism reduces and incorporates CO_2. Photosynthesis is one of the most significant metabolic processes on Earth because almost all our energy is ultimately derived from solar energy. It provides photosynthetic organisms with the ATP and reducing power necessary to synthesize the organic material required for growth. In turn these organisms serve as the base of most food chains in the biosphere. One type of photosynthesis is also responsible for replenishing our supply of O_2, a remarkable process carried out by a variety of organisms, both eucaryotic and bacterial. Although most people associate photosynthesis with the larger, more obvious plants, over half the photosynthesis on Earth is carried out by microorganisms. Photosynthesis as a whole is divided into two parts. In the light reactions light energy is trapped and converted to chemical energy. This energy is then used to reduce or fix CO_2 and synthesize cell constituents in the dark reactions.

Diversity of Phototrophic Organisms

Eucaryotic Organisms	Procaryotic Organisms
Plants	Cyanobacteria
Multicellular green, brown, and red algae	Green sulfur bacteria Green nonsulfur bacteria
Unicellular protists (e.g., euglenoids, dinoflagellates, diatoms)	*Halobacterium* (archaeon) Purple sulfur bacteria Purple nonsulfur bacteria *Prochloron*

BACTERIAL PHOTOSYNTHESIS

INTRODUCTION

Photosynthesis, the conversion of light energy to chemical energy, is one of the most important biological processes on earth. This process is characterized by two distinct set of reactions : the light reactions, in which light energy is conserved as chemical energy, and the dark reactions, in which this chemical energy is used to reduced carbon dioxide to organic compounds. Energy is supplied in the form of adenosine triphosphate (ATP) while electrons for the reduction of CO_2 come from NADH or NADPH (NADPH is the reduced substance in oxygenic photosynthesis

while NADH in anoxygenic photosynthesis). The latter is produced by the reduction of NAD+ or NADP+ respectively by electrons originating from various electron donor chemical compounds. The cyanobacteria use H_2O as a source of electron donor to reduce NADP+ to NADPH releasing molecular oxygen (O_2) as a by-product; this photosynthesis is called oxygenic. The phototrophic bacteria (purple and green bacteria) other than cyanobacteria use chemicals other than H_2O to donate electrons thus molecular oxygen (O_2) is not evolved as a by-product in their photosynthesis; this photosynthesis is called anoxygenic.

PHOTOSYNTHETIC STRUCTURES

In the eukaryotic cells of higher green plants, multicellualar red, green and brown algae, dinoflagellaies and diatoms the photosynthetic structures are the chloroplasts. Chloroplasts enclose membranous sacs called thylakoids which contain the units of photosynthesis. In prokaryotes (blue-green bacteria, Prochlorophyta, purple and green bacteria) the photosynthetic structures are chromatophores. In the Rhodospirillaceae (purple non-sulphur bacteria) and the Chromatiaceae (purple sulphur bacteria) the thylakoids are extensions of the cell membrane. They may be in the form of vesicles, tubular bodies or lamellae. In the Chlorobiaceae (green sulphur bacteria) the sacs forming the photosynthetic apparatus are not continuous with the cell membrane.

TYPES OF BACTERIAL PHOTOSYNTHESIS

Bacteria which use the radiant energy of light in nutrition are called phototrophs, while those utilizing chemical bond energy are called chemotrophs. Phototrophs normally utilize CO_2 as the source of carbon. The energy required for the reduction of CO_2 during its assimilation may be obtained from inorganic or organic substrates. Metabolism in which inorganic substances like H_2S or thiosulphate are the sources of reducing power is called photolithotrophy. When the oxidation of organic compounds like malate and succinate yields reducing power, the process is called photoorganotrophy. The general equation for pbotosynthesis is :

$$2\ H_2A + CO_2 \rightleftharpoons C(H_20) + H_2O + 2\ A$$

In green plants growing aerobically in light H_2A is H_2O. In photosynthetic bacteria that are obligatory anaerobes H_2A is hydrogen sulphide (H_2S) or organic molecules. In the Chromatiaceae and Chlorobiaceae the reducing substrate for replacing electrons are hydrogen sulphide or thiosulphate. In the Rhodospirillaceae the substrates are organic molecules like malate or succinate. Many members of the Rhodospirillaceae can grow in the dark. They apparently obtain their energy by electron Transport dependent phosphorylation linked to oxidation of organic molecules. An outline of the different types of reactions in bacterial photosynthesis is given below:

(i) Purple sulphur bacteria, e.g. Chromalium, utilize H_2S as the reducing substrate instead of H_20 in photosynthesis. Sulphur (S) is produced instead of oxygen.

$CO_2 + 2\ H_2S \longrightarrow C(H_20) + 2S + H_2O$

(ii) Purple sulphur bacteria can also use thiosulphate as a reductant

$2\ CO_2 + Na_2S_20_3 + 5H_2O \longrightarrow 2\ C(H_20) + 2\ H_20 + 2\ NaHSO_4$

(iii) In non-sulphur bacteria like Rhodo spirillum rubrum, the electron donors are organic compounds like ethanol, isopropanol of succinate,

$CO_2 + 3C2H_5OH \longrightarrow C\ (H_2O) + CH_3CHO + H_2O$

It will be seen that in green plants there is evolution of oxygen during photosynthesis, while in bacteria no oxygen is evolved.

The cyanobacteria differ from purple and green bacteria in the nature of their photosynthetic pigment system and in their ability for oxygenic photosynthesis.The purple and green bacteria carry out anoxygenic photosynthesis, i.e. there is no evolution of oxygen. There is only one photosystem involved in photosynthesis. The electron donors are sulphur, reduced sulphur compounds, molecular hydrogen or simple organic compounds. These are substunces with lower redox potentials than water. It should be noted that even in cyanobacteria there may be anoxygenic photosynthesis with only one photosystem when H_2S is the electron donor.

Oxygenic and anoxygenic photosynthesis: In the Cyanobacteria and Prochlorophyta photosynthesis is oxygenic, i.e. there is evolution of oxygen. There are two linked photosystems involved in photosynthesis. The electron donor is H_2O, and oxygen is the ultimate product of oxidation. They are, therefore, aerobic phototrophis. The photosynthetic apparatus of the Cyanobacteria is remarkably similar in structure and function to the eukaryote chloroplast. Their light harvesting pigments, Chl a and phycobiliproteins, are homologous to those of the chloroplast of Rhodophyta (red algae).

PHOTOSYNTHESIS PIGMENTS: There are three main classes of photosynthesis pigments, chlorophylls (Chl) (including bacteriochlorophylls, BChl), carotenoids and phycobilins (phycobiliproteins: PBPs). All species capable of carrying out photosynthesis contain one or more types of chlorophyll or bacteriochlorophyll and carotenoids. Phycobilins are found only in the red algae (Rhodophyta : eukaryotes) and the blue green bacteria (Cyanobacteria : prokaryotes). All phototrophic bacteria, except the Cyanobacteria, contain bacteriochlorophylls and carotenoids.

Chi c. Brown algue contain a compound called Chlc which is related to chlorophyll. Chl d has been reported in the red algae.

CHLOROPHYLLS AND BACTERIOCHLOROPHYLLS

All oxygenic phototrophic bacteria called cyanobacteria possess chlorophyll as the principal photosynthetic pigment. Chlorophyll is a porphyrin, containing a magnesium atom at the centre

of the porphyrin ring. There are different types of chlorophylls present in phototrophic bacteria; the principal chlorophyll of cyanobacteria is chlorophyll a as shown in

Structure of chlorophyll: The emperical formula of chlorophyll a is C_{55} $H_{72}0_5N_4Mg$. Chlorophyll a is a blue-green microcrystalline solid, consisting of a 'head' and a 'tail. The head consists of a porphyrin ring or tetrapyrrole nucleus, from which extends a tail made up of a 20-carbon grouping called the phytol. The porphyrins are complex carbon- nitrogen molecules that usually surround a metal. In chlorophyll the porphyrin surrounds a magnesium ion while in haemoglobin it surrounds an iron ion, The cytochromes of the electron transport system also have porphyrio rings. The basic unit of the porphyrin ring is the porphobilinogen molecule. Four such pyrroles make up units of the tetrapyrrole structure, Phytol is a long straight-chain alcohol containing a double bond. It may be regarded as a hydrogenated carotene (vitamin A), Its formula is $C_{20}H_{39}$

Chlorophyll b has the emperical formula $C_{55}H_{70}O_6N_4Mg$, It is a green-black microcrystalline solid. It differs from chlorophyll a in having an aldehyde (CHO) group attached to carbon atom 3, instead of a methyl (CH_3) group

Contrary to it, all anoxygenic phototrophs (purple bacteria, *i.e., purple sulphur bacteria* and *purple nonsulphur bacteria*, and *green sulphur bacteria*) have adopted bacteriochlorophyll instead of chlorophyll. Like chlorophylls, bacteriochlorophylls are also of different types (*a, b, c, c_s, d, e* and *g*). Bacteriochlorophylls are identical to chlorophylls and differ from it as well as from each other in possessing different substituents present in the positions R_1 to R_7 (Fig. 7.1 (B) and Table 7.1). For convenience, the structure of bacteriochlorophyll *a* is given in Fig. 7.1 (C).

Substituents of different Bacteriochlorophylls

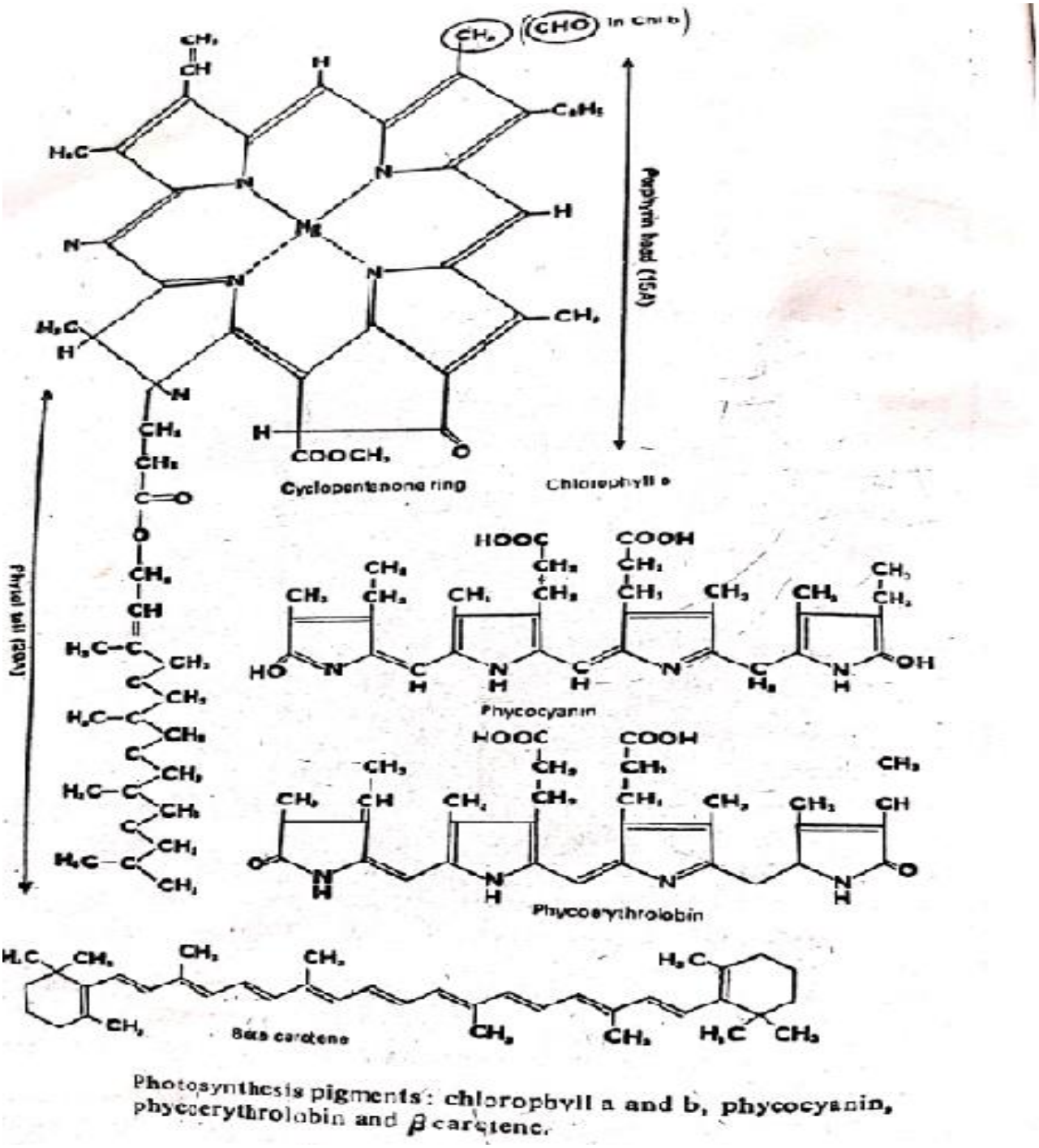
Bacteriochlorophylls	Substrates							Absorption spectra (nm)
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	
Bacteriochlorophyll *a* (purple bacteria)	$-\overset{\overset{O}{\|}}{C}-CH_3$	$-CH_3$[b]	$-CH_2-CH_3$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	P/Gg[a]	—H	830–890
Bacteriochlorophyll *b* (purple bacteria)	$-\overset{\overset{O}{\|}}{C}-CH_3$	$-CH_3$[c]	$=\overset{\overset{H}{\|}}{C}-CH_3$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	P	—H	1020–1040
Bacteriochlorophyll *c* (green sulfur bacteria)	$-\overset{\overset{OH}{\|}}{\overset{\overset{H}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$[d] $-C_4H_9$	$-C_2H_5$ $-CH_3$	—H	F	$-CH_3$	745–755
Bacteriochlorophyll c_s (green nonsulfur bacteria)	$-\overset{\overset{OH}{\|}}{\overset{\overset{H}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$	$-CH_3$	—H	S	$-CH_3$	740
Bacteriochlorophyll *d* (green sulfur bacteria)	$-\overset{\overset{OH}{\|}}{\overset{\overset{H}{\|}}{C}}-CH_3$	$-CH_3$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$ $-CH_3$	—H	F	—H	705–740
Bacteriochlorophyll *e* (green sulfur bacteria)	$-\overset{\overset{OH}{\|}}{\overset{\overset{H}{\|}}{C}}-CH_3$	$-\overset{\overset{O}{\|}}{C}-H$	$-C_2H_5$ $-C_3H_7$ $-C_4H_9$	$-C_2H_5$	—H	F	$-CH_3$	719–726
Bacteriochlorophyll *g* (heliobacteria)	$-\overset{H}{\|}C=CH_2$	$-CH_3$[b]	$-C_2H_5$	$-CH_3$	$-\overset{\overset{O}{\|}}{C}-O-CH_3$	F	—H	670, 788

[a]P, Phytyl ester ($C_{20}OH_{39}O-$); F, farnesyl ester ($C_{15}H_{25}O-$); Gg, geranylgeraniol ester ($C_{10}H_{17}O-$); S, stearyl alcohol ($C_{18}H_{37}O-$).

[b]No double bond between C_3 and C_4; additional H atoms are in positions C_3 and C_4.

[c]No double bond between C_3 and C_4; an additional H atom is in position C_3.

[d]Bacteriochlorophylls *c, d,* and *e* consist of isomeric mixtures with the different substituents on R_3 as shown.

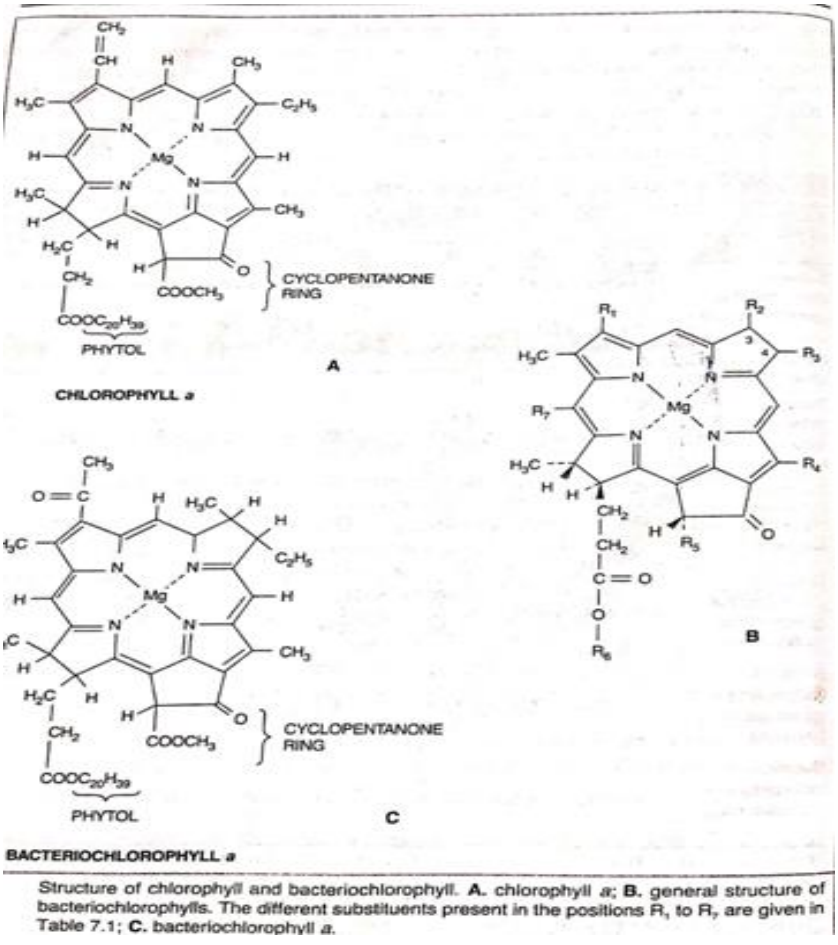


Photosynthesis pigments: chlorophyll a and b, phycocyanin, phycoerythrobilin and β carotene.

Bacteriochlorophylls: The photosynthetic pigments of the purple and green bacteria are bacteriochlorophylls a, b, c, d or e and a variety of carotenoids. The characteristics of the different bacteriochlorophylls are

Types of Bacteria Chlorophylls.

Designation of Jenson et. al, 1964	Former designation	Characteristic adsorption maxima in living cells (nm)
BChl *a*	Bacteriochlorophyll	375, 590, 805, 830–890
BChl *b*	Bacteriochlorophyll b	400, 605, 835–850, 1020–1040
BChl *c*	Chlorobium chl 660	Long wavelength absorption max 745–755
BChl *d*	Chlorobium chl 650	—do— 705–740



Structure of chlorophyll and bacteriochlorophyll. **A.** chlorophyll *a*; **B.** general structure of bacteriochlorophylls. The different substituents present in the positions R_1 to R_7 are given in Table 7.1; **C.** bacteriochlorophyll *a*.

In addition to chlororphyll or bacteriochlorophyll which are obligatory for photosynthesis, rious accessory pigments, mainly the carotenoids and the phycobiliproteins, occur in ototrophic microorganisms and are involved in the capture and processing of light

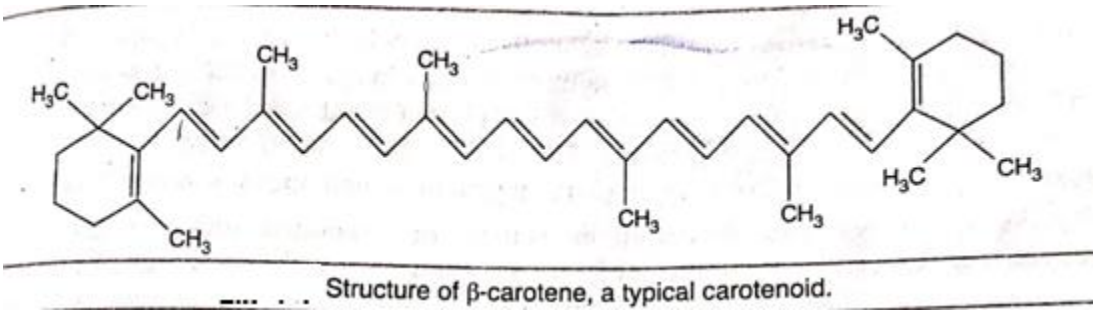
energy wever, the carotenoids play primarily a photoprotective role whereas the phycobiliproteins action in light harvesting.

Photosynthesis Pigments

GROUP	Chl/BChl a b c d	Carotenoids	PBPs
Eukaryotes			
Mosses, ferns, seed plants	Chl a b — —	+	—
Green algae	Chl a b — —	+	—
Euglenoids	Chl a b — —	+	—
Diatoms	Chl a — c —	+	—
Dinoflagellates	Chl a — c —	+	—
Brown algae	Chl a — c —	+	—
Red algae	Chl a — — d	+	+
Prokaryotes			
Blue-green bacteria	BChl a - - -	+	+
Purple sulphur bacteria	BChl a/b - -	+ Groups 1,2,4	—
Purple non-sulphur bacteria	BChl a/b - -	+ Groups 1,2,4	—
Green bacteria	BChl a - c/d	+	—

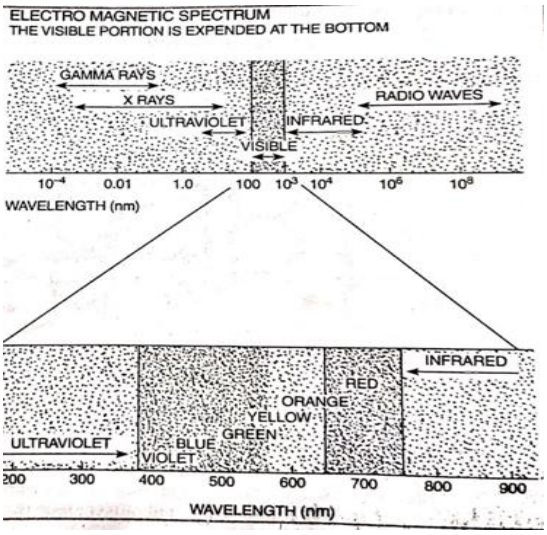
ACCESSORY PIGMENTS: CAROTENOIDS AND PHYCOBILIPROTEINS

Carotenoids are the most frequently occurring accessory pigments in ototrophic microorganisms. They are hydrophobic pigments firmly embedded in the embrane and are yellow, red, brown or green in colour. They absorb light in the blue region the spectrum (470-500 nm). Carotenoids remain closely associated with chlorophyll bacteriochlorophyll the photosynthetic membrane but do not function directly in photophosphorylation reactions; they act antenna chlorophylls/ bacteriochlorophylls and transfer the captured energy to the reaction centre where it is used in photophosphorylation. Carotenoids also function as photoprotective agents. Often, the bright light causes photooxidation reactions in cells resulting in the production of toxic oxygen which may bring destruction of the photosynthetic apparatus of the cell. Carotenoids absorb much of this harmful light and quench toxic oxygen.



Structure of β-carotene, a typical carotenoid.

Phycobiliproteins are the principal light harvesting pigments of cyanobacteria. They are openchain tetraphyrroles coupled to protein and are red or blue in colour. The red pigment called phycoerythrin harvests light most strongly at wavelengths around 550 nm, whereas the blue pigment called phycocyanin captures Light most strongly at 620 nm. Allophycocyanin, a third pigment, absorbs light at about 650 nm. Phycobiliproteins aggregate in the form of phycobilisomes, which is attached to the photosynthetic membranes. In phycobilisomes, the phycoerythrin and phycocyanin absorb light of shorter wavelengths (higher energy) and transfer it to allophycocyanin. The latter is closely linked to the reaction centre chlorophyll ‘a' and transfers energy to it, Cyanobacteria grow at fairly low light intensities due to the phycobilisome because the latter yields very efficient energy transfer to chlorophyll 'a' even at the lowest light intensities.



PHOTOSYNTHETIC BACTERIA

There are three groups of photosynthetic bacteria: the purple bacteria, the green bacteria, and the cyanobacteria. The cyanobacteria differ most fundamentally from the green and purple photosynthetic bacteria in being able to carry out oxygenic photosynthesis. They use water as an electron donor and generate oxygen during photosynthesis. In contrast, purple and green bacteria

use anoxygenic photosynthesis. Because they are unable to use water as an electron source, they employ reduced molecules such as hydrogen sulfide, sulfur, hydrogen, and organic matter as their electron source for the generation of NADH and NADPH. Consequently, purple and green bacteria do not produce oxygen but many form sulfur granules. Purple sulfur bacteria accumulate granules within their cells, whereas green sulfur bacteria deposit the sulfur granules outside their cells. The purple nonsulfur bacteria use organic molecules as an electron source. There also are differences in photosynthetic pigments, the organization of photosynthetic membranes, nutritional requirements, and oxygen relationships. The connection between photosynthetic pigments, oxygen relationships, and ecological distribution should be noted here.

Characteristics of the Major Groups of Photosynthetic Bacteria

	Anoxygenic Photosynthetic Bacteria				*Oxygenic Photosynthetic Bacteria*
Characteristic	**Green Sulfur[a]**	**Green Nonsulfur[b]**	**Purple Sulfur**	**Purple Nonsulfur**	**Cyanobacteria**
Major photosynthetic pigments	Bacteriochlorophylls *a* plus *c*, *d*, or *e* (the major pigment)	Bacteriochlorophylls *a* and *c*	Bacteriochlorophyll *a* or *b*	Bacteriochlorophyll *a* or *b*	Chlorophyll *a* plus phycobiliproteins
Morphology of photosynthetic membranes	Photosynthetic system partly in chlorosomes that are independent of the plasma membrane	Chlorosomes present when grown anaerobically	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Photosynthetic system contained in spherical or lamellar membrane complexes that are continuous with the plasma membrane	Membranes lined with phycobilisomes
Photosynthetic electron donors	H_2, H_2S, S	Photoheterotrophic donors—a variety of sugars, amino acids, and organic acids; photoautotrophic donors—H_2S, H_2	H_2, H_2S, S	Usually organic molecules; sometimes reduced sulfur compounds or H_2	H_2O
Sulfur deposition	Outside of the cell		Inside the cell[c]	Outside of the cell	
Nature of photosynthesis	Anoxygenic	Anoxygenic	Anoxygenic	Anoxygenic	Oxygenic (sometimes facultatively anoxygenic)
General metabolic type	Obligately anaerobic photolithoautotrophs	Usually photoheterotrophic; sometimes photoautotrophic or chemoheterotrophic (when aerobic and in the dark)	Obligately anaerobic photolithoautotrophs	Usually anaerobic photoorganoheterotrophs; some facultative photolithoautotrophs (in dark, chemoorganoheterotrophs)	Aerobic photolithoautotrophs
Motility	Nonmotile; some have gas vesicles	Gliding	Motile with polar flagella; some are peritrichously flagellated	Motile with polar flagella or nonmotile; some have gas vesicles	Nonmotile or with gliding motility; some have gas vesicles
Percent G + C	48–58	53–55	45–70	61–72	35–71

[a]Characteristics of *Chlorobi*.
[b]Characteristics of *Chloroflexus*.
[c]With the exception of *Ectothiorhodospira*.

The purple and green bacteria differ from the cyanobacteria in having bacteriochlorophylls rather than chlorophyll a. This proves to be quite useful because of the distinctive absorption spectra of the bacteriochlorophylls and accessory pigments. Normally green and purple bacteria are anaerobic and use H_2S and other reduced electron donors during photosynthesis. Because these bacteria grow best in deeper anaerobic zones of aquatic habitats, they cannot effectively use parts of the visible spectrum normally employed by photosynthetic organisms. There often is a dense surface layer of cyanobacteria and algae in lakes and ponds that absorbs a large amount of blue

and red light. The bacteriochlorophyll pigments of purple and green bacteria absorb longer wavelength, far-red light not used by other photosynthesizers.

Procaryotic Bacteriochlorophyll and Chlorophyll Absorption Maxima

Pigment	*Long Wavelength Maxima (nm)* In Ether or Acetone	Approximate Range of Values in Cells
Chlorophyll *a*	665	680–685
Bacteriochlorophyll *a*	775	850–910 (purple bacteria)[a]
Bacteriochlorophyll *b*	790	1,020–1,035
Bacteriochlorophyll *c*	660	745–760
Bacteriochlorophyll *d*	650	725–745
Bacteriochlorophyll *e*	647	715–725

[a]The spectrum of bacteriochlorophyll *a* in green bacteria has a different maximum, 805–810 nm.

In addition, the bacteriochlorophyll absorption peaks at about 350 to 550 nm enable them to grow at greater depths because shorter wavelength light can penetrate water farther. As a result, when the water is sufficiently clear, a layer of green and purple bacteria develops in the anaerobic, hydrogen sulfide-rich zone . The second edition of Bergey's Manualplaces photosynthetic bacteria into six major groups.

The phylum Chloroflexi contains the green nonsulfur bacteria, and the phylum Chlorobi, the green sulfur bacteria. The cyanobacteria are placed in their own phylum, Cyanobacteria. Purple bacteria are divided between three groups. Purple sulfur bacteria are placed in the γ -proteobacteria, families Chromatiaceae and Ectothiorhodospiraceae. The purple nonsulfur bacteria are distributed between the α-proteobacteria (five different families) and one family of the β-proteobacteria. The purple photosynthetic bacteria Phylum Chloroflexi The phylum Chloroflexi has both photosynthetic and nonphotosynthetic members. Chloroflexus is the major representative of the photosynthetic green nonsulfur bacteria. It is a filamentous, gliding, thermophilic bacterium that often is isolated from neutral to alkaline hot springs where it grows in the form of orange-reddish mats, usually in association with cyanobacteria. Although it resembles the green bacteria in ultrastructure and photosynthetic pigments, its metabolism is more similar to that of the purple nonsulfur bacteria. Chloroflexus can carry out anoxygenic photosynthesis with organic compounds as carbon sources or grow aerobically as a chemoheterotroph. It doesn't appear closely related to any bacterial group based on 16S rRNA studies and is a deep and ancient branch of the bacterial tree. Nutritional types. The nonphotosynthetic, gliding, rod-shaped or filamentous bacterium Herpetosiphon also is included in this phylum. Herpetosiphon is an aerobic chemoorganotroph with respiratory metabolism and oxygen as the electron acceptor. It can be isolated from freshwater and soil habitats.

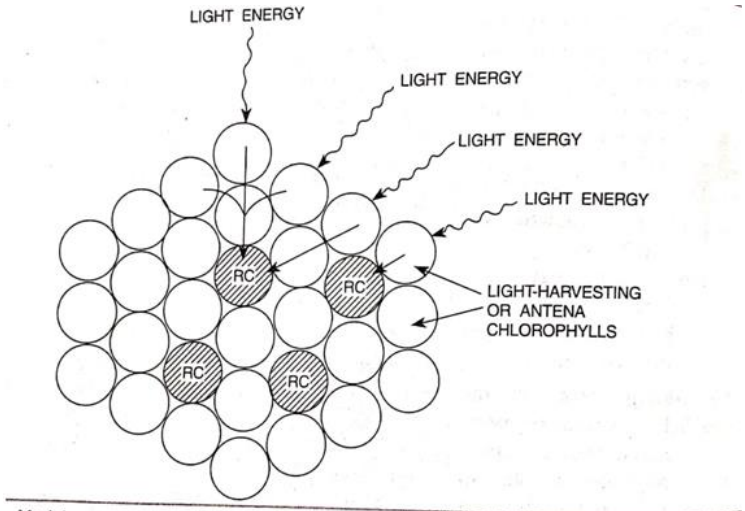
Phylum Chlorobi :The phylum Chlorobi has only one class (Chlorobia), order (Chlorobiales), and family (Chlorobiaceae). The green sulfur bacteria are a small group of obligately anaerobic photolithoautotrophs that use hydrogen sulfide, elemental sulfur, and hydrogen as electron sources. The elemental sulfur produced by sulfide oxidation is deposited outside the cell. Their photosynthetic pigments are located in ellipsoidal vesicles called chlorosomes or chlorobium vesicles,which are attached to the plasma membrane but are not continuous with it. The chlorosome membrane is not a normal lipid bilayer or unit membrane. Chlorosomes contain accessory bacteriochlorophyll pigments, but the reaction center bacteriochlorophyll is located in the plasma membrane and must be able to obtain energy from chlorosome pigments. These bacteria flourish in the anaerobic,sulfide-rich zones of lakes. Although they lack flagella and are nonmotile, some species have gas vesicles (figure 21.5a) to adjust their depth for optimal light and hydrogen sulfide. Those forms without vesicles are found in sulfide-rich muds at the bottom of lakes and ponds. The green sulfur bacteria are very diverse morphologically. They may be rods, cocci, or vibrios; some grow singly, and others form chains and clusters (figure 21.5b,c). They are either grass-green or chocolate-brown in color. Representative genera are Chlorobium, Prosthecochloris, and Pelodictyon.

Cyanobacteria The cyanobacteria are the largest and most diverse group of photosynthetic bacteria. There is little agreement about the number of cyanobacterial species. Older classifications had as many as 2,000 or more species. In one recent system this has been reduced to 62 species and 24 genera. The second edition of Bergey's Manual of Determinative Bacteriologydescribes 56 genera in some detail. The G C content of the group ranges from 35 to 71%. Although cyanobacteria are true procaryotes, their photosynthetic system closely resembles that of the eucaryotes because they have chlorophyll a and photosystem II, and carry out oxygenic photosynthesis. Like the red algae, cyanobacteria use phycobiliproteins as accessory pigments. Photosynthetic pigments and electron transport chain components are located in thylakoid membranes lined with particles called phycobilisomes(figure 21.6). These contain phycobilinpigments, particularly phycocyanin,and transfer energy to photosystem II. Carbon dioxide is assimilated through the Calvin cycle,and the reserve carbohydrate is glycogen. Sometimes they will store extra nitrogen as polymers of arginine or aspartic acid in cyanophycin granules. Since cyanobacteria lack the enzyme -ketoglutarate dehydrogenase,they do not have a fully functional citric acid cycle. The pentose phosphate pathway plays a central role in their carbohydrate metabolism. Although many cyanobacteria are obligate photolithoautotrophs, some can grow slowly in the dark as chemoheterotrophs by oxidizing glucose and a few other sugars. Under anaerobic conditions *Oscillatoria limnetica* oxidizes hydrogen sulfide instead of water and carries out anoxygenic photosynthesis much like the green photosynthetic bacteria. As these examples illustrate, cyanobacteria are capable of considerable metabolic flexibility.

PHOTOSYNTHETIC APPARATUSES AND REACTION CENTRES: In eukaryotic microorganisms, there are chloroplasts (special intracellular/organelles) that function as photosynthetic apparatus. The chlorophyll pigments are attached to lamellar (sheet-like)

membrane structures of the chloroplast called thylakoids. The stacks of thylakoids are called grana. Contrary to it, the chloroplasts are absent in prokaryotic microorganisms and the photosynthetic pigments remain integrated into internal membrane system. The latter arise (i) from invagination of the plasma membrane (purple bacteria), (ii) in both the plasma membrane and specialized non-unit membrane-enclosed structures called chlorosomes (green sulphur bacteria), or (ii) in thylakoid membranes (cyanobacteria).

Chlorophyll or bacteriochlorophyll molecules are associated with proteins to form complexes consisting of anywhere from 50 to 300 molecules. Only a very small number of these pigment molecules participate directly in the conversion of light energy to chemical energy (ATP), and are called reaction centre chlorophylls or bacteriochlorophylls .



. Model representing the arrangement of light harvesting chlorophylls/bacteriochlorophylls versus reaction centres within a photosynthetic membrane. RC = reaction centre

The latter are surrounded by the more numerous light-harvesting or antenna chlorophylls or Bacteriochlorophylls. The antenna pigments capture light and transfer the energy of light to the reaction centre.

Unlike purple bacteria wherein the bacteriochlorophyll molecules are associated with instead function much like a solid state circuit. They absorb light of low intensities and funnel the energy of light to reaction centre bacteriochlorophyll. Bucause of having ability to capture very low intensity light with the help of chlorosomes, the green sulphur bacteria can grow at the lowest light intensity of any known phototrophic microorganism.

OXYGENIC PHOTOSYNTHESIS

Light Reaction in Cyanobacteria: In cyanobacteria (also in all phototrophic eukaryotes), there are two distinct but interconnected photosystems : photosystem I and photosystem II Photosystem I absorbs longer wavelength light (far-red light) and funnels its energy to a special reaction centre chlorophyll ‘a” molecule called P_{700} .The P_{700} signifies that this reaction centre

chlorophyll 'a' absorbs light at a wavelength of 700 nm most effectively. Photosystem II absorbs light at shorter wavelengths (near red light) and transfer its energy to the reaction centre chlorophyll molecules called P_{680}

Cyclic Photophosphorylation :When the photosystem I antenna chlorophylls funnel light energy to the reaction centre chlorophyll P700, the latter gets excited and, as a result, its reduction potential becomes very negative. The excited or high-energy electron of P700 is captured by a special chlorophyll 'a' molecule (A) or an iron sulphur protein (FeS). The electron is eventually transferred to ferredoxin. The latter transfers electron to a cyclic route through a series of electron carriers (cytochrome b_{563} -------- plastaquinone-------cytochrome b_6------ cytochrome f -------plastocyanin) back to oxidized P_{700}. Since the electrons travel in a cyclic pathway (i.e. they originate from P_{700} and come back to the P_{700}), the process is called cyclic photophosphorylation in which only photosystem I is involved. During cyclic phosphorylation, ATP is generated in the region of cytochrome b_6

Non-Cyclic Photophosphorylation: In this photophosphorylation both photosystem I and II are involved. The reduction potential of P_{680} chlorophyll a molecule of photosystem II is very electropositive, slightly more positive than that of the $H_20/0_2$ couple. This facilitates the first step in oxygenic electron flow, the splitting of water (photolysis) into oxygen atoms ($1/20_2$) and hydrogen ions ($2H^+$). Photolysis donates an electron to the oxidized P_{680} molecule following the absorption of a quantum of light near 680 nm. The P_{680} molecule is now excited and reduces pheophytin 'a' which is chlorophyll ‘a' without the magnesium atom. Electrons susbequently travel through quinone, plastaquinone, cytochrome b_6 (ATP is generated in the region of cytochrome b_6), cytochrome f and plastocyanin; the latter donates electrons to photosystem I. The electron is accepted by the oxidized reaction centre chlorophyll ‘a’ of photosystem I (P_{700}) which has previously absorbed light quanta and begun the steps to lead the reduction of $NADP^+$ into NADPH.

ANOXYGENIC PHOTOSYNTHESIS

Purple and green bacteria possess only photosystem I. Since they lack photosystem II, they cannot use water (H20) as an electron donor in noncyclic photophosphorylation (ie., noncyclic electron transport) and thus cannot produce oxygen from water photosynthetically, i.e., they are anoxygenic.

Light Reaction in Purple Bacteria: Light-harvesting antenna bacteriochlorophyll molecules absorb light and transfer it to reaction centre bacteriochlorophyll called P_{870}. P_{870} is excited and releases electron which proceeds to reduce a molecule of bacteriopheophytin (Bph) in the reaction centre. This transition completes very fastly taking about three-trillionth of a second (i.e., 10^{-12} sec.) time. Once reduced, the bacteriopheophytin reduces several intermediate quinone (O molecules to finally, a quinone in "quinone pool". This transition is also very fast completing within less than one-billionth of a second. Electrons are now transported from the quinone

through a series of iron-sulphur proteins (FeS) and cytochromes (Cyt) back to the reaction centre (P_{870}). It is the cytochrome bc_1 complex that interacts with the quinone pool during photosynthetic electron flow as a proton motive force (PMF) used to derive ATP synthesis. In addition to ATP, NADP or NADPH are also produced by purple bacteria using H S (also $S_20_3^{2-}$, S^0 and even $Fe^{2+)}$ as external electron donors. When H_2S is the electron donor, globules of sulphur (S^0) are stored inside the cells of purple bacteria.

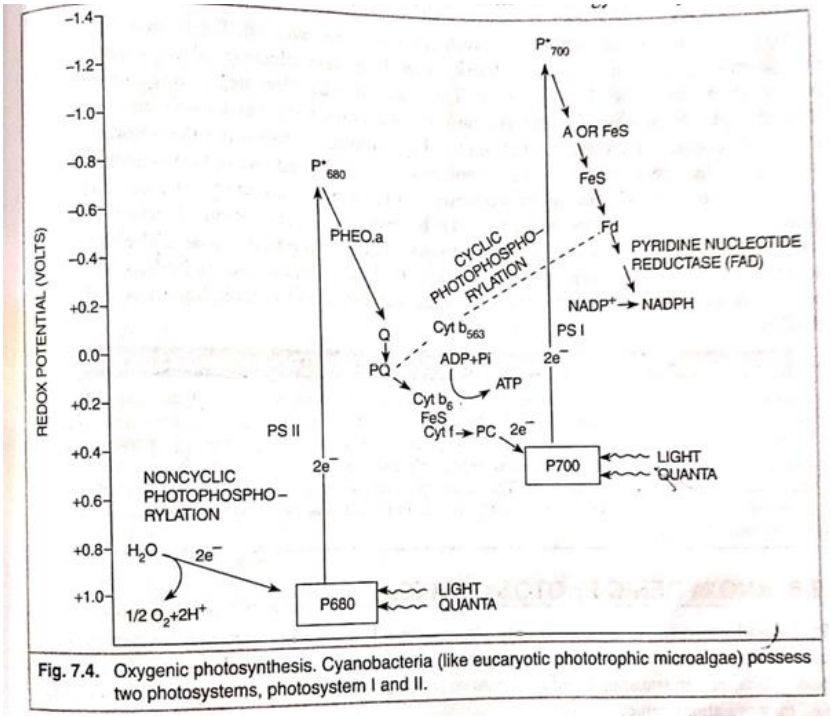
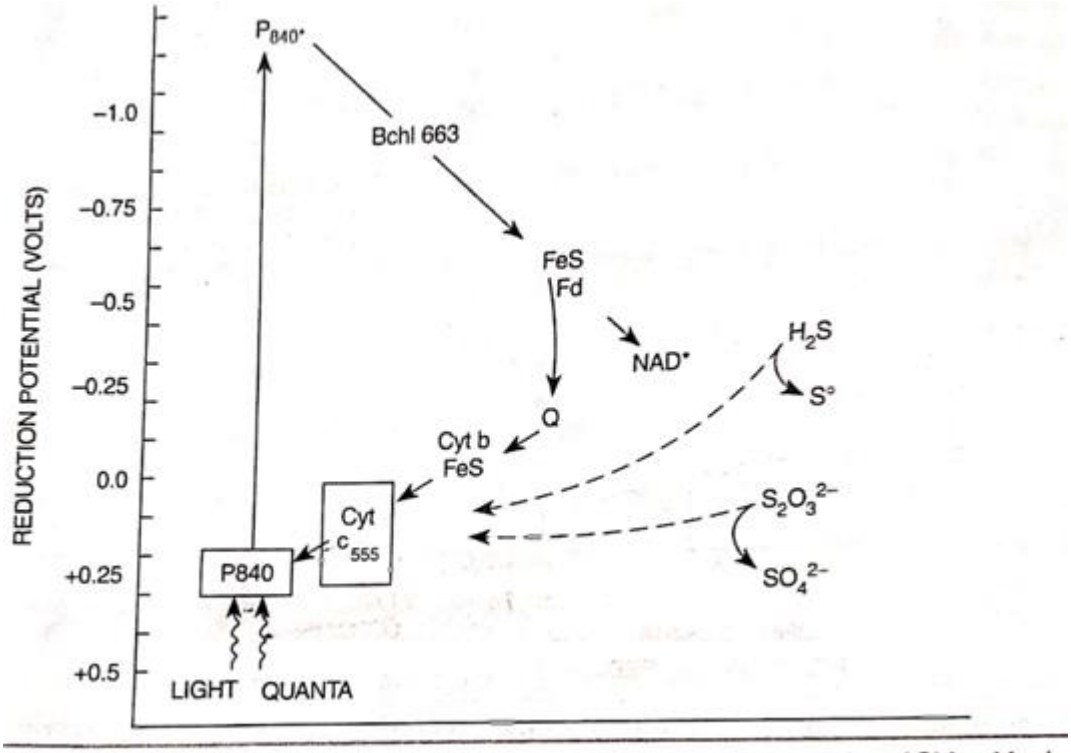


Fig. 7.4. Oxygenic photosynthesis. Cyanobacteria (like eucaryotic phototrophic microalgae) possess two photosystems, photosystem I and II.

A reversed electron flow operates in purple bacteria to reduce NAD* to NADH. The reduced H_2S or H_2SO^{2-} (thiosulphate) are oxidized by cytochromes and electrons from them eventually end up in quinone pool. However, the energy potential of quinone is insufficiently negative to reduce NAD^+ directly. Therefore, the electrons from the quinone pool are forced backward to reduce NAD^+ to NADH. This energy requiring process is called reversed electron flow.

Light Reaction in Green Bacteria: The general scheme of cyclic photophosphorylation is represented in Fig



Scheme of electron flow (cyclic photophosphorylation) in green bacterium (*Chlorobium*).

The reaction centre bacteriochlorophyll is P_{840} that it absorbs light near 840 nm and resides at a significantly more negative reduction potential in comparison to purple bacteria. Unlike purple bacteria where the first stable electron acceptor molecule resides at about 0.0 reduction potential, the electron acceptors of green bacteria (FeS proteins) reside at about - 0.6 reduction potential and have a much more electronegative reduction potential than NADH. In green bacteria, ferredoxin reduced by FeS protein serves directly as electron donor for dark reaction (fixation of CO_2) Thus, like oxygenic phototrophic microorganisms (and even green plants), in green bacteria both ATP and NADPH are direct products of light reactions. When H_2S donates electrons to reduce NAD to NADH in green bacteria, sulphur globules remain outside of the cell of green bacteria. This is unlike purple bacteria where the globules of sulphur remain inside of the bacterial cell.

Summarized Account of Important Differences in Photosynthetic Systems of Microbes

	Eukaryotic microalgae and cyanobacteria	Green and purple bacteria
(*i*) Photosynthetic pigment	Chlorophyll	Bacteriochlorophyll
(*ii*) Photosystem II	Present	Absent
(*iii*) Photosynthetic electron donors	H_2O	Inorganic (H_2S, H_2, S etc) and organic matter
(*iv*) Pattern of oxygen production	Oxygenic[1]	Anoxygenic
(*v*) Source of carbon	CO_2	CO_2 and/or organic matter
(*vi*) Primary products of energy conversion	ATP + NADPH	ATP

1 = Some cyanobacteria (*e.g.*, *Oscillatoria limmetica*) and microalgae use only photosystem I under certain conditions and do not produce oxygen hence behave anoxygenically.

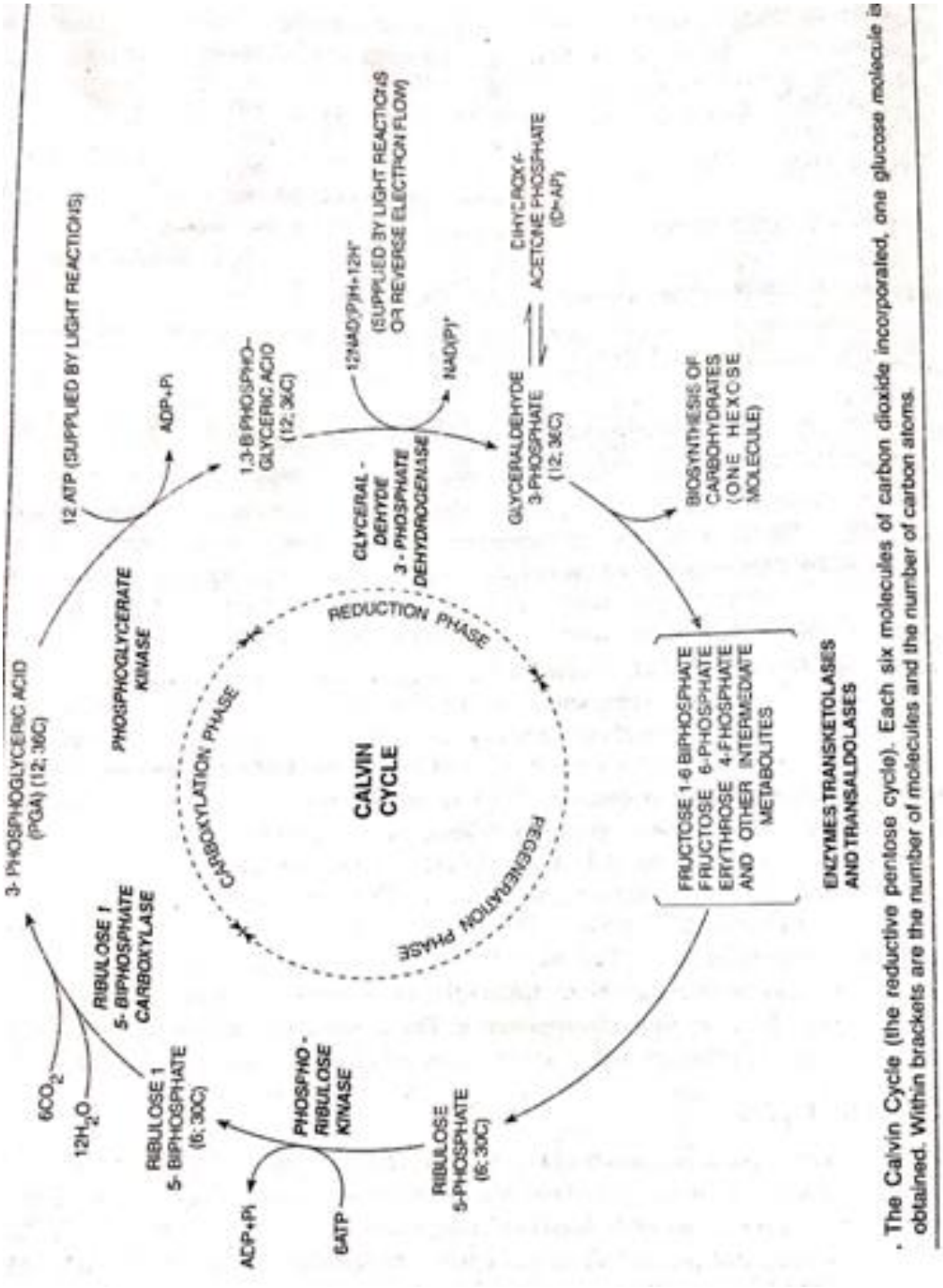
FIXATION OR ASSIMILATION OF CO_2 IN MICROORGANISMS : THE DARK REACTION

Although most microorganisms can fix or assimilate carbon dioxide (CO_2), only autotrophic ones use CO_2 as their sole or principal carbon source. The reduction or assimilation of CO_2, takes place at the expense of much energy. Usually autotrophic microorganisms obtain the required energy by trapping light during photosynthesis (photoautotrophs), but some derive it from the oxidation of reduced inorganic electron donors (chemoautotrophs).

Microorganisms can fix CO_2 or convert this inorganic molecule to organic carbon and assimilate it in certain major ways, which are the Calvin cycle (also called Calvin-Benson cycle, or reductive pentose phosphate pathway), the reductive tricarboxylic acid cycle (also called reductive TCA cycle, or reverse citric acid cycle), the hydroxypropionate cycle or acetyl-CoA pathway. Carbon dioxide (CO_2) is incorporated by almost all microbial autotrophs using Calvin cycle, a special metabolic pathway. Although this cycle in most photosynthetic microorganisms, it is absent in archaea (archaebacteria), some obligately anaerobic bacteria, and some microaerophilic bacteria. These microorganisms usually use rest of the two above mentioned pathways. The reductive tricaboxylic cycle is used by some archaea (archaebacteria), e.g., Thermoproteus, Sulfolobous and by bacteria such as Chlorobium, a green sulphur bacterium. Chloroflexus, a green non-sulphur photoautotroph uses the unique pathway of hydroxypropinonate. The acetyl CoA pathway is employed by methanogens, sulphate reducers, and acetogenes (bacteria that can form acetate from CO_2).

Calvin Cycle

Phototropic microorganisms (microalgae, cyanobacteria, purple and green bacteria), like plants, assimilate CO_2 to produce carbohydrate principally through Calvin cycle. The latter is named for its discover Melvin Calvin and is also popular by the names CalvinBensen cycle or reductive pentose phosphate cycle. The Calvin cycle requires NAD(P)H and ATP and two key enzymes, ribulose-1, 5-biphosphate carboxylase (ribulose bisphosphate carboxylase) and phosphoribulokinase. To understand the Calvin-cycle easily, it can be divided into three phases (carboxylation, reduction, and regeneration).



. The Calvin Cycle (the reductive pentose cycle). Each six molecules of carbon dioxide incorporated, one glucose molecule is obtained. Within brackets are the number of molecules and the number of carbon atoms.

The Carboxylation Phase: During this phase of co, pivation the eneme blow hiphosphate care worse or ribulour bisphosphate barn (in short form o..... RUBISCO) catalyzes the incorporation of co to Hibulose 1 sbphosphate (RAP) to penerate two molecules of 3-phosphoglyceric acid (IGA)

The Reduction Phase: 1-phosphophyceric acid (PA) is produced to glyceraldehyde phosphate with the envolvement of two enzymes. Phosphaghirate kiwe chayme Rothen 3-phosphoglycerie acid into 1, 3-byphosphate glveeric acid which is then weduwed to glyceraldehyde 3-phosphate by enzyme chveraldehide phosphate dehwimween

The Regeneration Phase: During this phase the ribulose I. 5 biphosphate (RunP') in regenerated and carbohydrates such as truecose and glucose are produced, Cilyconaldehyle 3-phosphate is converted to dihydroxyacetone phosphate (DHAP'); this conversion is reversible. Most of these twoje glyceraldehyde phosphate and DHAP are used to regenerate ribulose-1. S-biphosphate via various intermediate steps involving transkotolane and transaldolase reactions, the remaining ones are used in the biosynthesis of carbohydraten,

The stoichiometry of the Calvin cycle can be represented in tow words as 12 NADPH and I8ATP are required to synthesize 1 hexose molecule (glucose) from 6 molecules of CO_2. The overall equation can be summarized as under

$6CO_2$ + I8ATP + 12NADPH + $12H^+$ + $12H_2$ 0 ---------- 1 Hexose + 18ADP +18Pi+ $12NADP^+$

Reductive Tricarboxilic Acid Cycle (Reduced TCA Cycle): The reductive tricarboxylic acid cycle (reduced TCA cycle, reduced Kreb's cycle, or reduced citric acid cycle), which is also called reverse TCA cycle, is used as alternative mechanism of CO, fixation by phototrophite green sulphur bacteria (e.g., Chlorobium) and by some nonphototrophic archaebacteria (Thermoproieus, Sulfolobus and Aquifex). In this cycle, the CO2 fixation takes place by a reversal of steps in the tricarboxylic acid cycle (a major pathway of respiration by which pyruvate is completely oxidised to CO2). In Chlorobium, there are two ferredoxin-linked enzymes that catalyse the reductive fixation of CO2 into intermediates of the tricarboxylic acid cycle. The two ferredoxin-linked reactions involve the carboxylation of succinyl-CoA to a-ketoglutarate and the carboxylation of acetyl-CoA to pyruvate

The reductive tricarboxylic acid cycle starts from oxaloacetate and each complete turn of the cycle results in three molecules of CO2 being incorporated and pyruvate as the product. All reactions of the cycle are catalysed by enzymes of normal tricarboxylic acid cycle, but they work in reverse. One exception is citrate lyase, an ATP-dependent enzyme that cleaves citrate into acetyl-CoA and oxaloacetate in green sulphur bacteria. Citrate lyase replaces citrate synthetase that produces citrate from oxaloacetate and acetyl-CoA in the normal TCA cycle. However, the acetyl-CoA of reduced TCA cycle produces pyruvate, which is converted to phosphoenolpyruvate that then results in triose-phosphate. The triose-phosphate converts into hexose-phosphate (glucose-phosphate), which is utilised in cell material.

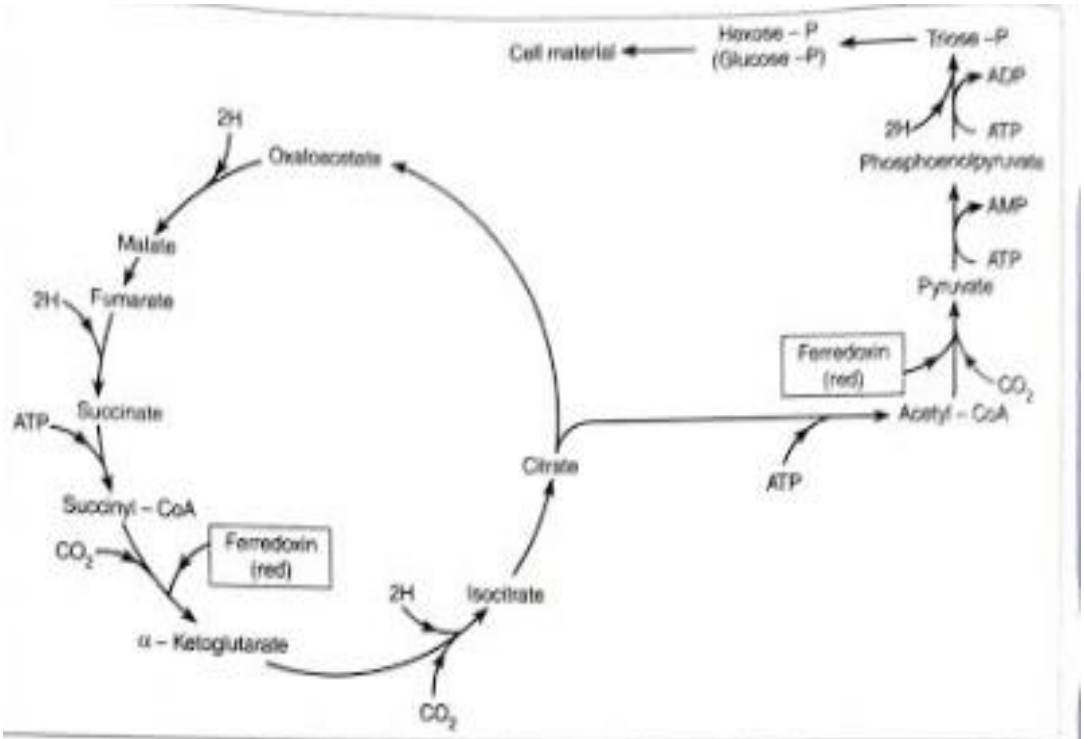


Fig. 7.8. The reductive (or reverse) tricarboxylic acid cycle

Hydroxypropionate Pathway: Hydroxypropionate pathway is also a mechanism of autotrophic Co, fixation unique to green nonsulphur bacteria (Chloroflexus). Choroflexus, an anoxigenic photoautotroph, uses either H_2 or H_2S as electron donors. In hydroxypropionate pathway, two molecules of CO_2 are reduced to glyoxylate. Acetyl-CoA is carboxylated to yield methylmanonyl-CoA. This intermediate is rearranged to yield acetyl-CoA and glycoxylate. The latter is converted to cell material.

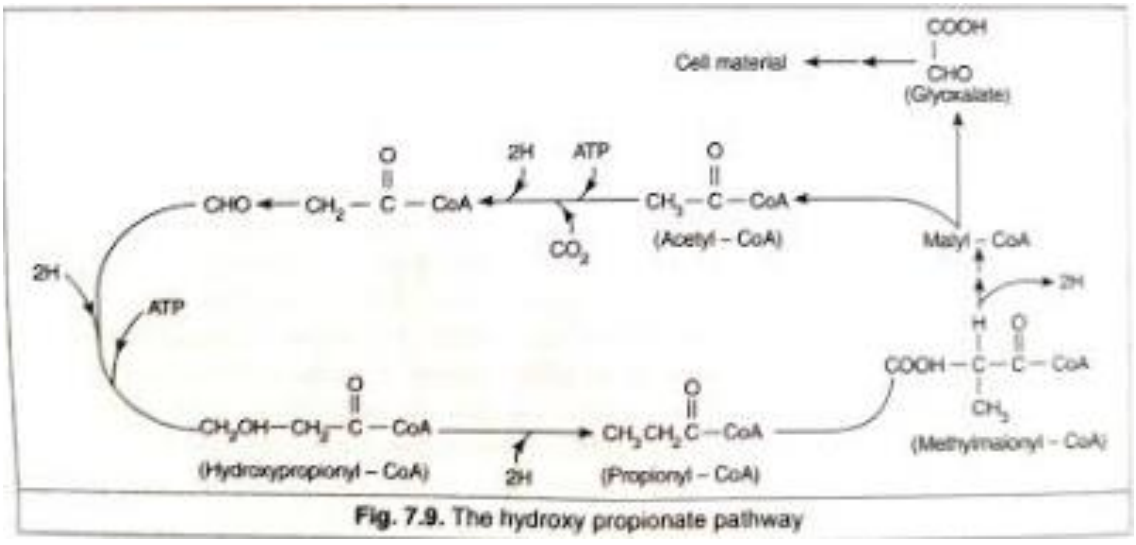


Fig. 7.9. The hydroxy propionate pathway

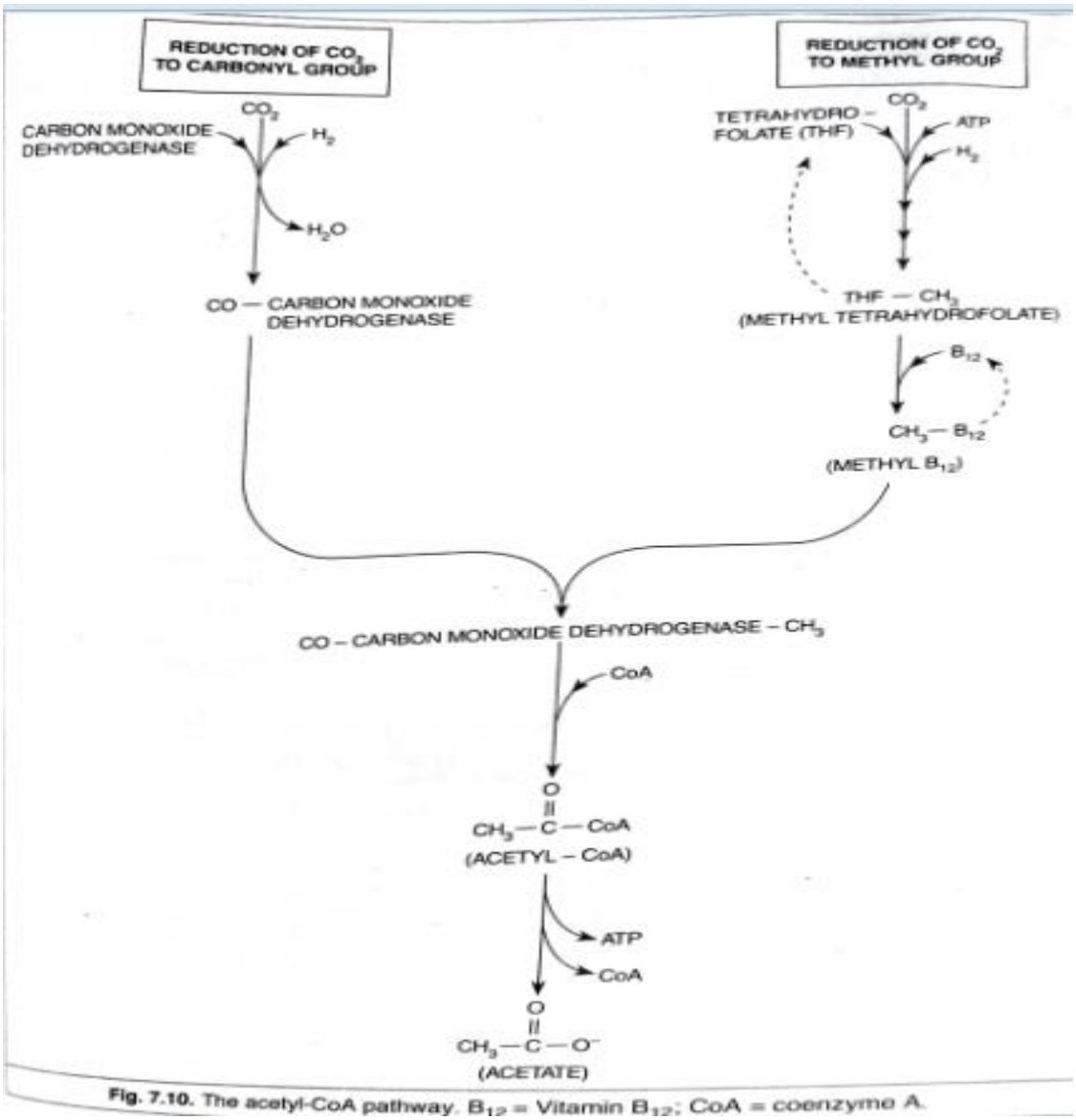


Fig. 7.10. The acetyl-CoA pathway. B_{12} = Vitamin B_{12}; CoA = coenzyme A.

Hydroxypropionate pathway has so far been confirmed only in Chloroflexus and appears to be of evolutionary significance. Chloroflexus is a "hybrid" photoautotroph in the sense that its photosynthetic mechanism shows features characteristic of both purple sulphur bacteria and green sulphur bacteria. Bacteriochlorophyll a located in the cytoplasmic membrane of cells of Chloroflexus is arranged to form a photosynthetic reaction centre structurally similar to those of purple bacteria. Chloroflexus, on the other hand, contains bacteriochlorophyllc and chlorosomes (oblong bacteriochlorophyll-rich bodies bound by a thin, nonunit membrane lying attached to the cytoplasmic membrane in the periphery of the cell) like green sulphur bacteria. It has thus been proposed that modern Choloroflexus may be a vestige of a very early phototrophic ancestor that perhaps first evolved a photosynthetic reaction centre and then received chlorosome-specific genes by lateral transfer.

The Acetyl-CoA Pathway: The acetyl-CoA pathway (Fig. 7.10) is employed by methanogenic archaebacteria (methanogens), sulphate-reducing bacteria, and acetogenic bacteria (acetogenes). The acetylCoA pathway of CO_2 assimilation is not cyclic like other CO_2 assimilative pathways. Instead it involves the fixation of CO_2 via two linear pathways. One molecule of CO_2 is reduced to the methyl group of acetate, and the other molecule of CO_2 is reduced to the carbonyl group. These two linear pathways assemble at the end to form acetyl-CoA, which then is carboxylated resulting in pyruvate. The key enzyme of acetyl-CoA pathway is carbon monooxide dehydrogenase (CO dehydrogenase) that contains nickle (Ni), zinc (Zn), and iron (Fe) as cofactors, Carbon monoxide (CO) produced by the reaction catalysed by this enzyme ends up in the carbonyl (- COO-) position of acetate. The methyl group of acetate originates from the reduction of Co, by a series of reactions involving the coenzyme tetrahydrofolate (THF). The methyl group is then transferred from tetrahydrofolate (THF) to an enzyme containing vitamin B12 as cofactor. The methyl group (CH_3 group) combines with CO in CO-dehydrogenase to form the final product, acetyl-CoA.