

## Photosynthesis and Biosynthesis

As described earlier, **chemoheterotrophs** obtain the energy they need for growth from the catabolism of preformed organic compounds. These organisms make ATP using either **substrate level** or **oxidative phosphorylation** (or a combination of the two). Organisms categorized as **phototrophs** can make use of a third mechanism for synthesizing ATP called **photophosphorylation**. These organisms use light as their energy source.

### Photophosphorylation:

**Photophosphorylation** is ATP synthesis involving light (usually sunlight) as the energy source. Organisms capable of photophosphorylation carry one or more types of light-trapping pigments, i.e., molecules that can respond to light energy in a specific manner. In the case of **algae, cyanobacteria** and green plants, the pigments involved are green **chlorophyll** molecules (chlorophyll a, b or c); in the case of **anoxygenic phototrophic bacteria** they are **bacteriochlorophylls** (bacteriochlorophyll a, b, c, d, or e), which occur in a variety of colors; in the case of **Archaea** in the genus *Halobacterium* the pigment is **bacteriorhodopsin**, a red or purple-colored integral protein. All of these pigment molecules have a feature in common, and that is their ability to respond to light energy. Chlorophylls and bacteriochlorophylls respond by passing energy and eventually by passing electrons to electron acceptor molecules, while bacteriorhodopsin responds by changing configuration and “pumping” hydrogen protons across the cell membrane.

Light has both wave-form and particle-form properties, so although light occurs as different wavelengths, it also occurs as particles or packets of light energy called **photons**. When photons strike the **bacteriorhodopsin** molecules of *Halobacterium salinarum*, these function as light driven “proton pumps” that transport hydrogen protons across the cell membrane (out of the cell or into the periplasmic space). The accumulation of hydrogen protons creates a concentration and electrical gradient (the **proton motive force**), and this causes a return flow of protons through **ATP-synthase**. The resulting formation of ATP from ADP and  $\text{PO}_4$  is one example of photophosphorylation.

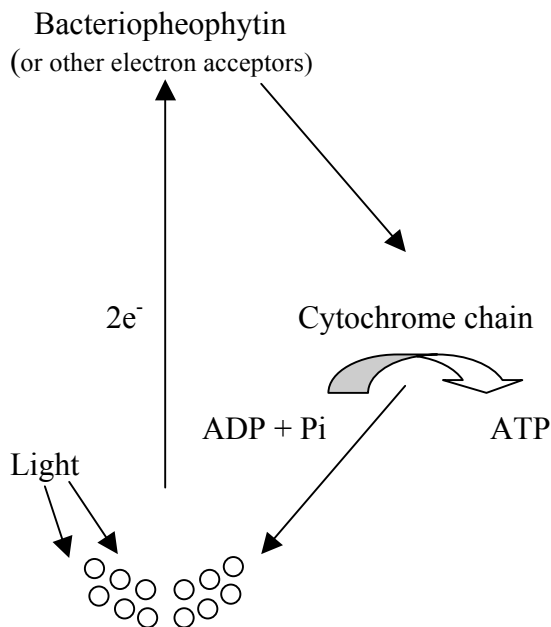
When photons strike chlorophylls or bacteriochlorophylls, these molecules can pass energy from one to another, or certain atoms within them can give up electrons, i.e., pass electrons to other molecules. If the electrons are passed to proteins associated with a membrane-bound electron transport chain or system (integral proteins), their flow can trigger the same reactions we saw associated with **oxidative phosphorylation**, i.e., the transport of hydrogen protons across the membrane to establish a **proton motive force**, and the return flow of protons through **ATP-synthase** resulting in the formation of ATP. Again the ATP made would be the result of photophosphorylation.

Not all of the light energy captured by phototrophs is used to make ATP. Some of it is used to reduce the coenzyme NADP to  $\text{NADPH} + \text{H}^+$ . Recall that the reduced form of a coenzyme has a higher energy potential than does the oxidized form. Because this energy capture does not involve ATP, it is not photophosphorylation.

Photophosphorylation can be described as **cyclic** or **non-cyclic** on the basis of whether or not the electrons leaving the pigment molecules return to them (or to other pigment molecules of the same type). During **cyclic photophosphorylation**, the electrons return to the pigment molecules (i.e., they are recycled), while during **non-cyclic photophosphorylation**, they do not. When pigment molecules give up electrons during non-cyclic photophosphorylation, these must be replaced, and this can result in the formation of a very important compound.

## Photophosphorylation in Anoxygenic Phototrophs:

**Anoxygenic phototrophs** include the green and purple sulfur bacteria, green and purple non-sulfur bacteria and other phototrophic prokaryotes not included in the phylum Cyanobacteria. These organisms are called **anoxygenic** (an = without, oxy = oxygen and genic = generation or production of), because they do not produce oxygen in association with photophosphorylation. Cyclic photophosphorylation as it occurs in these organisms can involve different pigment molecules (alone or in combination), but in general they are all **bacteriochlorophylls**. These respond to light energy by passing electrons to an electron acceptor e.g., **ubiquinone**, **bacteriopheophytin**, or others. From the acceptor molecules the electrons travel along a series of cytochromes, and then back to the pigments. The diagram shown below is a simplified representation of the pathway taken by activated electrons during **cyclic photophosphorylation**.



In this diagram light is striking pigment molecules and causing electrons associated with certain atoms to gain energy. Electrons can then either pass energy (via resonance) to electrons of other molecules (the antenna array), or "bounce" away to an atom in some other molecule (sometimes these travel in pairs as indicated here, and sometimes they do not). The electrons leave their pigment molecules at **reaction centers**, and are passed to some type of electron acceptor (in this case **bacteriopheophytin**). From there they can travel along a chain of **cytochrome** enzymes (via oxidation/reduction reactions), and supply the energy needed to "pump" hydrogen protons across the membrane. As electrons exit the cytochrome chain, they are picked up by atoms associated with pigment molecules (bacteriochlorophylls), and are

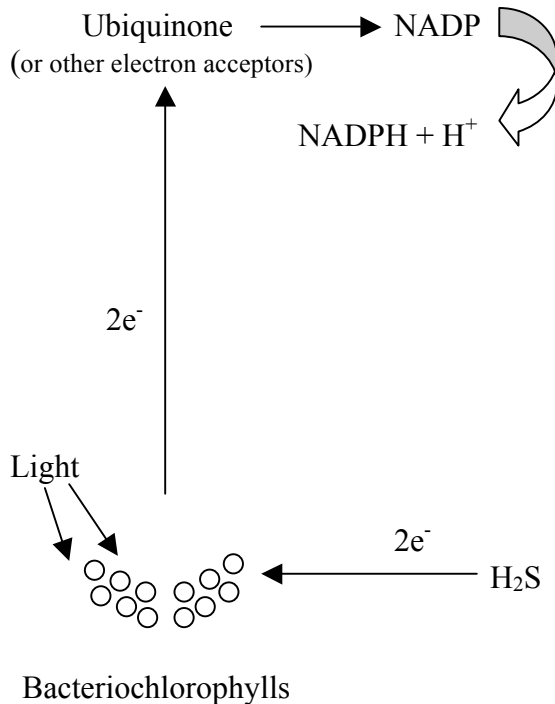
ultimately returned to the atoms they came from (or to atoms of pigments of the same type). The flow of electrons is associated with ATP synthesis as explained below. Since the electrons involved in this process are recycled, the synthesis of ATP can continue as long as light energy is available.

The pigment molecules, electron acceptors and cytochromes indicated above are all bound to the bacterial cell membrane, so this is where photophosphorylation occurs. The electron flow generated by light striking the pigment molecules is linked to the transport of hydrogen protons across the membrane and into the periplasmic space (as indicated above). The accumulation of protons outside the membrane creates a **concentration and electrical gradient** known as the **proton motive force** (again like water accumulated behind a dam). When the protons flow back across the membrane (down their concentration and electrical gradient), they pass through **ATP-synthase** (an enzyme complex) and supply the energy needed to convert ADP + Pi (inorganic phosphate) into ATP. As long as light is available, ATP can be generated.

Green and purple sulfur bacteria can also capture light energy by means of a **non-cyclic** pathway. In this case, the electrons leaving the pigment molecules pass to an electron acceptor and then to the coenzyme **NADP** reducing it to **NADPH + H<sup>+</sup>**. The reduced coenzyme has a higher energy potential than it does in its oxidized state, so some of the energy available in the light (photons) is captured, but the pigment molecules are now missing electrons. Bacteriochlorophylls cannot pull electrons and hydrogen protons away from water molecules, so oxygen is not generated, but these pigments can pull

electrons away from hydrogen sulfide, resulting in the formation of elemental sulfur. Bacteria samples taken from inside the Winogradsky window at the back of the laboratory often contain sulfur granules within their cytoplasm. These appear as tiny refractile particles readily visible when the bacteria are magnified 1000X with a light microscope.

The diagram shown below represents a **non-cyclic** pathway for capturing light energy as it occurs in green and purple sulfur bacteria.



In this diagram light is striking pigment (bacteriochlorophyll) molecules and causing the electrons from certain atoms to gain energy. Molecules in the antenna array pass energy, but at reaction centers, electrons “bounce” away (sometimes in pairs as shown here, and sometimes not). The electrons leaving pigment molecules then travel to an electron acceptor (in this case **ubiquinone** (also known as coenzyme Q), and from there to the coenzyme NADP, reducing it to NADPH + H<sup>+</sup>. Again we can note that NADPH + H<sup>+</sup> carries considerably more potential energy than does NADP just as NADH + H<sup>+</sup> carries more than NAD. In this case, the pigment molecules replace their missing electrons by pulling them away from hydrogen sulfide (H<sub>2</sub>S). Although this diagram looks similar to the one used to illustrate non-cyclic photophos-

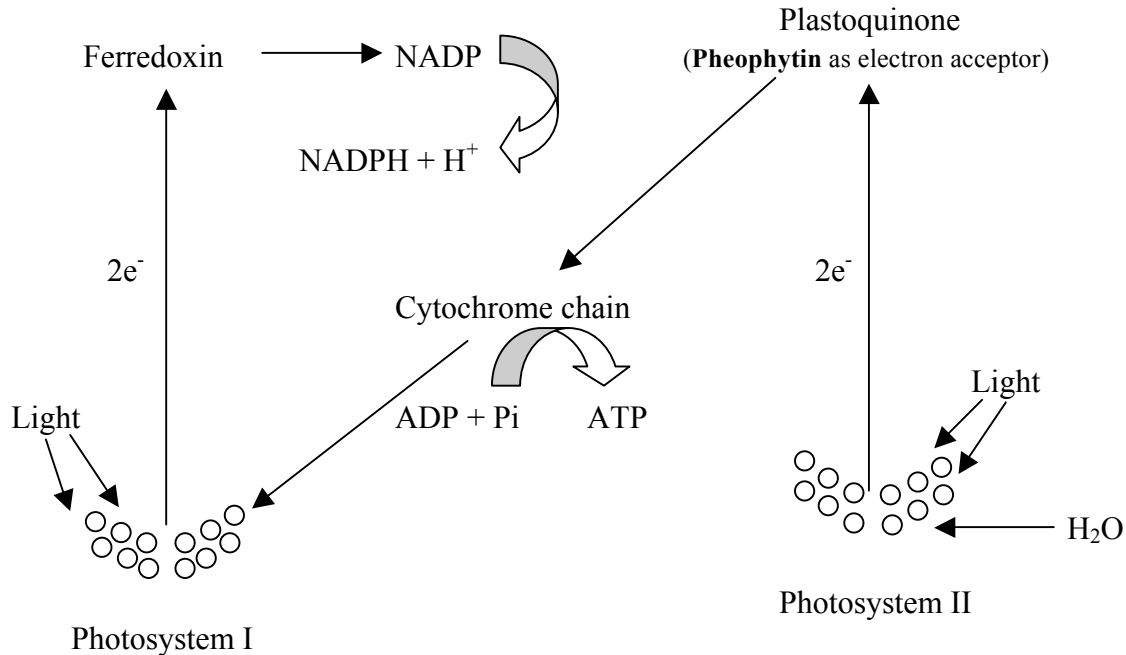
phorylation in algae and cyanobacteria (as described below), ATP is not being formed, so phosphorylation is not occurring. The anoxygenic phototrophic bacteria can capture light energy in the form of either ATP molecules or NADPH + H<sup>+</sup> molecules, but not in both at the same time. They can switch back and forth between the two mechanisms as required for maintaining other metabolic processes.

### Photophosphorylation in Algae and Cyanobacteria:

Algae and cyanobacteria (like green plants) are **oxygenic phototrophs**, i.e., organisms capable of generating molecular oxygen in association with their photophosphorylation processes. Like the green and purple sulfur bacteria described above, algae and cyanobacteria can use both cyclic and non-cyclic pathways, but since both of these generate ATP, both are truly photophosphorylation pathways.

Algae and cyanobacteria capture light energy with **chlorophyll pigments** (primarily) in a manner like that described above; however, pigments other than chlorophylls (e.g. carotenoids or phycobilins) can serve in the antenna array. Some of the electron acceptors associated with the **photosystems** (pigment systems) of oxygenic organisms are also different (plastoquinone, ferredoxin and pheophytin). In addition to this, algae and cyanobacteria typically use two different sets (accumulations) of proteins and pigment molecules capable of responding to different wavelengths of light. These two systems are called **photosystem I** (pigment system I) and **photosystem II** (pigment system II). When both photosystems are involved, photophosphorylation is non-cyclic and energy is captured in the formation of both ATP and NADPH + H<sup>+</sup>. When only one photosystem is being used (photosystem I), photophosphorylation is cyclic, and only ATP is generated.

The diagram below shows a highly simplified representation of **non-cyclic photophosphorylation** as it occurs in algae, cyanobacteria and green plants.



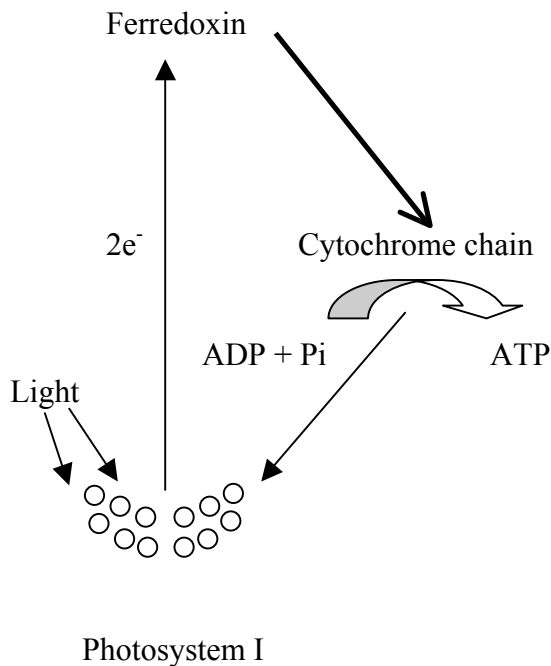
In this diagram, light is striking pigment molecules associated with both **photosystem I** and **photosystem II** and is causing electrons to leave certain atoms. The electrons leaving photosystem II are passed to the electron acceptor **pheophytin**, and then to **plastoquinone**. From Plastoquinone the electrons are passed along a chain of **cytochrome enzymes**, and are then transferred to photosystem I. At the same time, the electrons leaving photosystem I are passed to an electron acceptor called **ferredoxin** (an iron sulfur protein). From ferredoxin, the electrons pass to an enzyme complex, and then to **NADP**, reducing it to  $NADPH + H^+$ .

The proteins and pigments of photosystem I and II are bound to membranes called **thylakoids** (thylakos = sac). The passage of electrons along the chain of **cytochrome enzymes** is linked to the transport of hydrogen protons across these membranes (into the region surrounded by them). This creates a concentration and electrical gradient, i.e., a **proton motive force** that drives the passage of hydrogen protons through **ATP-synthase**, resulting in the conversion of  $ADP + Pi$  into  $ATP$ .

Since the electrons that leave photosystem II do not returned, the phosphorylation process is non-cyclic. Photosystem II can replace the lost electrons by pulling them away from water molecules, and thus generates **molecular oxygen** ( $2 H_2O$  minus 4 electrons and 4 hydrogen protons =  $O_2$ ).

The photosystems described above are actually very complex, each containing multiple proteins, light-trapping pigments and other types of molecules. In green algae and higher plants, most of the pigments associated with the photosystems are **chlorophylls**, but **carotenoid** pigments can also be involved. In red algae and cyanobacteria, chlorophylls are often accompanied by **phycobilins**. Though electrons are depicted as moving in pairs within the diagrams presented here, this is not always the case.

During **cyclic photophosphorylation** as it occurs in algae and cyanobacteria, electrons leave photosystem I, are passed to **ferredoxin**; however, rather than being passed to **NADP**, the electrons are passed to a chain of **cytochrome enzymes**, and then return to photosystem I.  $ATP$  is generated as described above, but oxygen is not produced. A simplified version of cyclic photophosphorylation is provided in the diagram below.

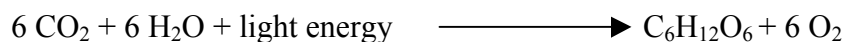


In this diagram, light is striking the pigment molecules of photosystem I and causing the electrons from some atoms to transfer energy to others. Eventually the electrons from some atoms gain enough energy to leave or “bounce” away (sometimes in pairs). The electrons travel to an electron acceptor, in this case an iron-sulfur protein called **ferredoxin**. From ferredoxin, the electrons pass to a chain of cytochrome enzymes (supplying energy needed to pump protons across the membrane), and from there, back to photosystem I. Since the electrons leaving the pigments of photosystem I will eventually be returned to the same photosystem, the process is cyclic. Note that during cyclic photophosphorylation energy is captured in the form of ATP, but not as NADPH + H<sup>+</sup>. In addition to this, there is no splitting of water molecules, and oxygen is not produced.

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### Photosynthesis:

**Photosynthesis** – Photosynthesis (photo = light, synthesis = building reactions) or light synthesis is often defined as a process allowing organisms to make sugar and oxygen using light energy, carbon dioxide and water. The chemical equation sometimes used to represent photosynthesis is shown below.



Although convenient, this equation is only partially correct, i.e., is only correct when applied to certain organisms. Algae, cyanobacteria and green plants produce oxygen in association with their photosynthetic processes, but anoxygenic phototrophs do not. In addition, although photoautotrophs use light energy and inorganic carbon as indicated by this equation, photoheterotrophs do not and neither do chemoautotrophs.

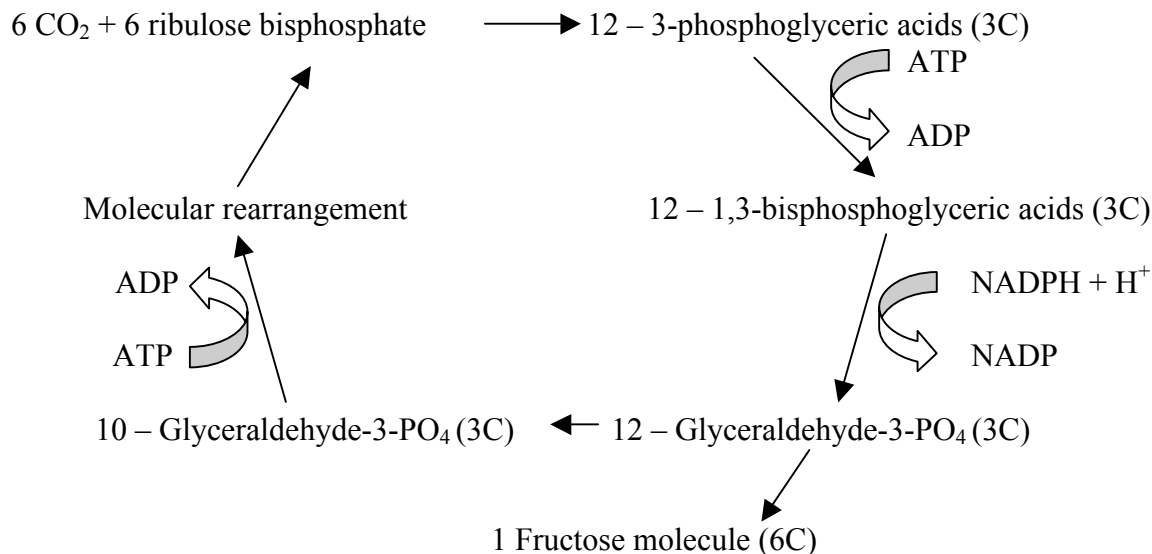
**Photosynthesis** is actually a complex metabolic process involving two distinctly different phases, these are:

- 1) **Photophosphorylation** (light dependent reactions) – As explained above, photophosphorylation involves making ATP using energy provided by light. When the process is non-cyclic and occurs within algae, cyanobacteria and green plants, it also results in the formation of NADPH + H<sup>+</sup>.
- 2) The **Calvin-Benson cycle** (light independent reactions) – An **anabolic pathway** used to convert inorganic carbon (carbon dioxide) into sugar (fructose). This pathway requires energy and is driven by the ATP and NADPH + H<sup>+</sup> generated in association with photophosphorylation.

As explained earlier, photophosphorylation may or may not result in the formation of oxygen. If it does, the oxygen is generated when water molecules donate electrons and hydrogen protons to photosystem II. The pigment systems of anoxygenic phototrophs do not split water and cannot generate oxygen.

## The Calvin-Benson Cycle:

The **Calvin-Benson cycle** is an **anabolic pathway** involving enzymes associated with the **stroma of chloroplasts** in eukaryotic cells (algae and green plants) and inclusions called **carboxysomes** in prokaryotes. One of the most important enzymes involved in the Calvin-Benson cycle is **ribulosebiphosphate carboxylase oxygenase (RuBisCO)**. This enzyme catalyzes the reaction binding **carbon dioxide** to **ribulose biphosphate**, a five-carbon sugar at the beginning of the cycle. As indicated above, the light independent reactions do not require light; they do, however, require energy. The ATP and NADPH + H<sup>+</sup> generated during photophosphorylation can be used to drive the Calvin-Benson cycle. A simplified version of the Calvin-Benson cycle is presented in the diagram below.



Six molecules of carbon dioxide are required to produce one molecule of sugar. Although fructose is formed initially, this can readily be converted to glucose (recall that fructose and glucose are isomers). The synthesis of fructose also requires two molecules of ATP and one molecule of NADPH + H<sup>+</sup> as shown above.

The Calvin-Benson cycle is **not** the only pathway autotrophs can use to "fix" carbon, i.e., to incorporate inorganic carbon into organic compounds. Furthermore, high-energy compounds generated through photophosphorylation processes do not necessarily drive the Calvin-Benson cycle. Chemoautotrophs can fix carbon, but do not have the photosystems necessary to capture light energy. Since we have already seen that some phototrophs do not produce oxygen (cannot split water), and since photoheterotrophs do not fix carbon at all, we can see that the chemical equation for photosynthesis is not always applicable.

## Alternate sources of energy and carbon:

As described earlier, chemoheterotrophs use carbohydrates (primarily glucose) as a major source of **energy**, i.e., they catabolize carbohydrates and use the energy released to form ATP. These organisms also use glucose as a major source of **carbon**. Many of the compounds formed during the catabolism of glucose, i.e., during glycolysis and the Krebs cycle, can also be used to form other compounds essential to cell function. As we shall see below, other compounds can also be catabolized and their by-products used as sources of both energy and carbon. When the catabolism of organic compounds is linked to the anabolism of other compounds (which within cells it often is), the overall process is called **intermediary metabolism**. Biochemical pathways used both in catabolism and in anabolism are called **amphibolic pathways**.

The reactions of glycolysis and the Krebs cycle are of considerable importance because many of the metabolic intermediates formed, e.g., Pyruvic acid, oxaloacetic acid and  $\alpha$ -ketoglutaric acid can have amino groups added to them to **form amino acids**, and these can be used to form proteins. The acetyl-CoA formed just prior to the Krebs cycle can donate two-carbon acetyl groups for use in the **synthesis of fatty acids**. The glyceraldehydes-3-phosphate formed during glycolysis is in equilibrium with dihydroxyacetone phosphate, and this can be converted into glycerol, the 3-carbon compound used in the **production of triglycerides and phospholipids**. Most of the nucleotides used to build nucleic acids (high-energy compounds, coenzymes and regulatory molecules) are obtained through the catabolism of nucleic acids ingested by cells (i.e., via **salvage pathways**); however, all types of cells can also **synthesize nucleotides** from components provided by amino acids and 5-carbon sugars.

### **Protein catabolism:**

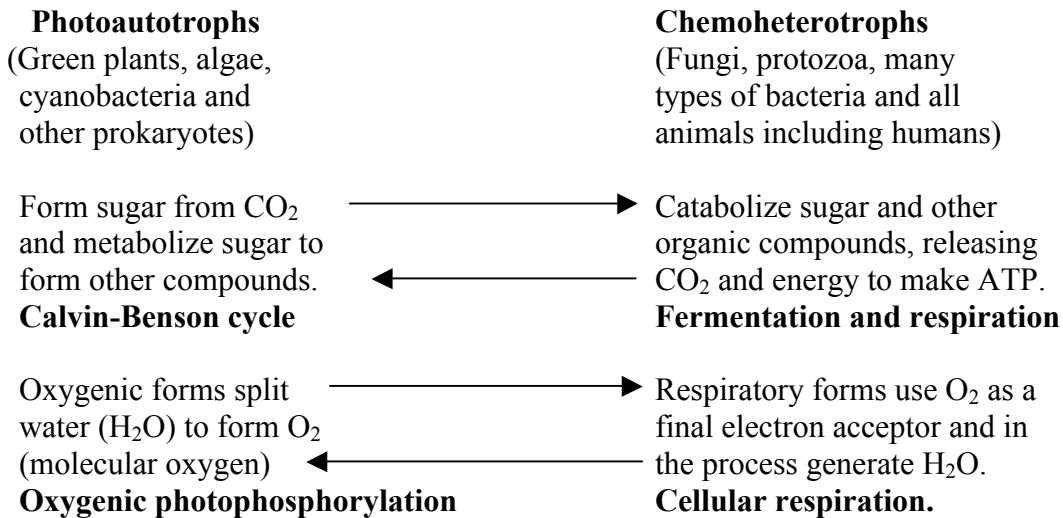
Chemoheterotrophs cultured in the microbiology laboratory are often provided with proteins or protein breakdown products (peptone, peptides, proteoses) as nutrient sources (recall that nutrient agar contains beef extract and peptone). Like carbohydrates, these nutrients can be catabolized to provide cells with both **energy and carbon**. Proteins can be broken into individual amino acids, and these can be used to build other (different) proteins (i.e., via salvage pathways). Amino acids can also be **deaminated**, i.e., can have their amino groups removed, and can then be completely catabolized through chemical reactions associated with glycolysis and/or the Krebs cycle depending on the type of amino acid initially present.

### **Lipid catabolism:**

Triglycerides and phospholipids are abundant within cells and can also be catabolized to yield both **energy and carbon**. The glycerol "backbone" associated with these molecules can be removed and phosphorylated to form dihydroxyacetone phosphate (DHAP). Since this molecule is in equilibrium with 3-phosphoglyceraldehyde (PGAL), it can readily enter the glycolysis pathway for additional catabolism. Fatty acids (hydrocarbon chains) can be broken into two-carbon units and oxidized through a process called **b-oxidation**. The resulting acetyl groups can then bind with **coenzyme-A** to form **acetyl-CoA**, and can then enter the Krebs cycle for additional processing.

## Ultimate symbiosis:

A more complete understanding of metabolic processes often leads to a greater appreciation for the **interdependence** of the organisms present on this planet. In a metabolic sense, all living organisms live **symbiotically** with other organisms, human beings included. **The random devastation of the natural environment perpetuated by human activities is ultimately self-destructive.** Without other life forms, we cannot survive. A brief summary of our obvious interdependence is illustrated in the diagram provided below.



Remember that although photoautotrophs use light as an energy source, they can only do this when light is available. These organisms use a respiratory type metabolism and consume organic compounds and oxygen just as we do. When light is available, they produce more oxygen and sugar than they consume, but during hours of darkness the reverse is true. Recall that **eutrophication** can lead to fish kills when algae use excessive amounts of oxygen in water during periods of darkness (e.g., at night). It is important to remember that the cycling of carbon into and out of organic compounds (carbon cycle), and the cycling of oxygen with water, represents only a fraction of the chemical processes involved in metabolism. Bacteria also play crucial roles in the cycling of nitrogen, sulfur, phosphorous, iron, and other elements essential to life.

## Global Warming and Other Important Issues – A Biological Perspective:

All life forms require a source of energy, and since energy cannot be created (produced) or destroyed, it flows through biological systems. Light energy (electromagnetic radiation from the sun) is the ultimate source of energy for organisms living on this planet. Only certain organisms can capture this energy and convert it to high-energy compounds available for metabolism (ATP and NADPH + H<sup>+</sup>); e.g., green plants, algae, cyanobacteria, certain other bacteria and a few types of archaea. These organisms have been capturing light energy for a very long time (millions of years at least). Some of the energy was used to drive metabolic processes within these organisms, and some was used to form organic compounds (cellular components for new cells). Eventually, geologic processes caused some cellular material to become buried under layers of sediments as deposits of coal and oil. Cellular respiration and fermentation allow organic compounds to be catabolized, often releasing CO<sub>2</sub> into the atmosphere, and these processes have also been occurring for a very long time (millions of years).

Fire, the non-biological conversion of organic compounds and oxygen into carbon dioxide and water has also been around as a natural phenomenon for millions of years (volcanoes, lightning, etc. are natural



sources of fire). Unfortunately, within the last few hundred years (due to the industrial revolution), human activities have significantly increased the rate at which buried organic materials are being burned. The increased production of water in association with this activity is hardly significant because  $\frac{3}{4}$  of the earth's surface is covered by water and the amount of liquid water present is influenced primarily by temperature fluctuations. The release of carbon dioxide is much more important. The burning of fossil fuels (coal and oil) in association with human activities (powering automobiles, planes, ships, etc., heating and cooling homes, offices, etc., generating electricity, driving industrial processes, etc.), has significantly increased carbon dioxide levels within our atmosphere. From a biological standpoint, increasing CO<sub>2</sub> levels can have catastrophic consequences.

Carbon dioxide is a "greenhouse" gas, i.e., a gas that tends to trap heat energy from the sun within the earth's atmosphere, rather than allowing it to radiate into space. Although warming the planet's surface is beneficial to some extent, too much heat is likely to have unpleasant consequences. **Global warming will potentially:**

- 1) Melt glacial ice, causing sea levels to rise and flooding much of the low-lying agricultural land currently available (imagine California's central valley filled with sea water).
- 2) Allow parasites and vectors to broaden their ranges and increase the incidence of diseases such as malaria, yellow fever, sleeping sickness, river blindness, schistosomiasis, etc. etc.
- 3) Cause organisms not adapted to warm environments to die. Bacteria are not the only organisms influenced by temperature, and many of the plant species currently inhabiting this planet will not survive significant increases in temperature. Loss of photoautotrophs means loss of oxygen production.
- 4) Disrupt normal weather patterns and increase the occurrence and severity of hurricanes, torrential rains, droughts and other natural phenomena recognized as being damaging to human endeavors.
- 5) Cause discomfort and stress within the human population, taxing medical facilities and creating unnecessary strife. The human population is already greater than can be sustained in the manner we are accustomed to. Imagine shrinking the landmasses available for food production, increasing damage due to natural phenomena, and increasing the ranges of vectors and pathogens known to transmit/cause human disease. **The impact on health-care facilities and personnel will be enormous.**

In addition to this, carbon dioxide reacts with water to form carbonic acid, and lowers the pH within water environments. Increases in atmospheric carbon dioxide levels are already causing damage in ocean environments where decreases in pH are killing corals and other organisms. Recall that pH has a significant impact on enzyme activity. Killing organisms in ocean environments can have severe consequences, especially if the organisms dying are oxygenic photoautotrophs (refer to diagram above if you don't remember why). Carbon dioxide is also used as the primary building material for methane within methanogenic prokaryotes. Methane released into the atmosphere also acts as a "green house" gas, compounding the potential for global warming.

### **What can be done?**

As explained during an earlier lecture, humans have tremendous intellectual potential, and if properly educated could eliminate many of the problems currently facing mankind. Relative to the issues described in this section, students can have a positive impact by:

- 1) **Voting responsibly** – Look at the issues, consider the consequences, and vote in a manner beneficial to all humans now and in the future.
- 2) **Taking responsibility for your actions** – Don't wait for someone else to solve the world's problems; take action yourself. Become informed, reproduce responsibly, recycle, eat more vegetables, plant trees, reduce your dependence on coal and oil, carpool, turn off the engine when the car is not in use, park it and walk, take a bus, etc., etc.

- 3) **Promoting education and new energy technology** – Coal, oil and nuclear power are potentially dangerous, and alternative energy sources (solar, wind, hydrogen, etc.) are available. Promote education and encourage the development/use of alternative, ecologically "friendly" energy sources.
- 4) **Spreading the word** – Tell other people; the microbiology web site is available to everyone, encourage other people to read it.

We are biological entities and live symbiotically with other biological entities in a complex ecosystem maintained by interactions we do not fully understand. Human activity **is** negatively impacting this ecosystem in ways that will ultimately be damaging to us; **our future is at stake**. Fortunately, we don't have to worry about the fate of microorganisms, they will be just fine.