

Biomass – Photosynthesis

4.1 PHOTOSYNTHESIS

4.1.1 Chemotrophs and Autotrophs

In the preceding sections we have studied the concept of metabolism and learnt that all living organisms require an external energy source. Using this criterion, all living organisms can be divided into two groups, those that use **chemical energy** and those that use **light energy**. The organisms that can use light as an energy source are called **phototrophs**, this group includes plants, algae and some bacteria. The process whereby this occurs is photosynthesis.

The organisms known as **chemotrophs** require a chemical energy source. This can be either inorganic (**chemolithotrophs**) or organic, as there are a few types of bacteria that can obtain energy from inorganic chemicals by oxidation/reduction processes. However, the majority of this group require an organic chemical source to provide energy for life-maintaining functions. Most bacteria fall into this group and, of course, all higher life forms such as animals and humans. This information is summarised below in **Figure 1**.

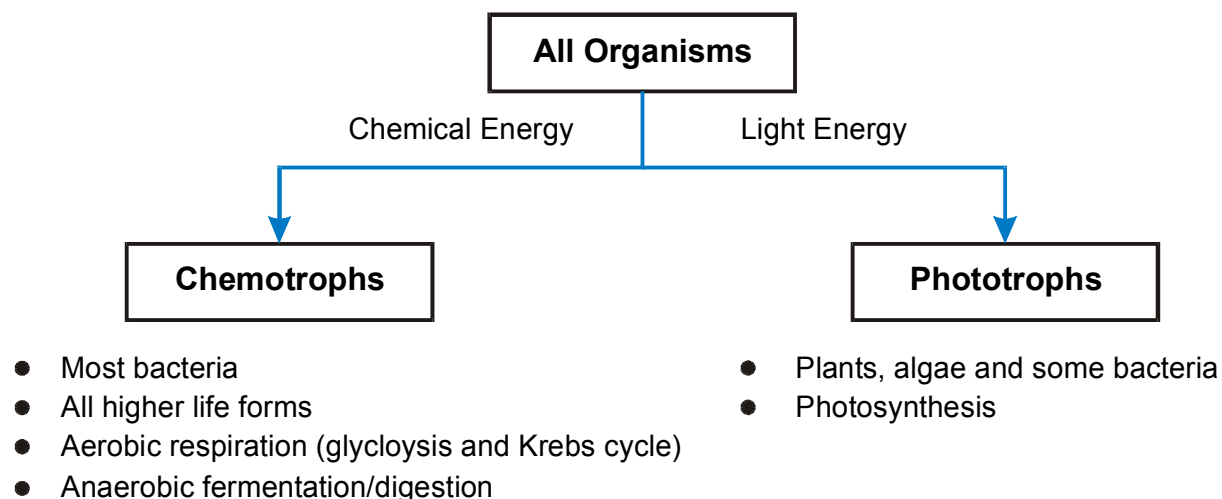


Figure 1. The characteristics of Chemotrophs and Phototrophs.

The diagram of **Figure 2**, shown overleaf, may look like identical to **Figure 1**, however, instead of chemotrophs and phototrophs we now have **heterotrophs** and **autotrophs**. These different terms are used because the emphasis is not on the energy source used but on the source of carbon required.

All living organisms are composed of a framework of carbon, which is obtained either from non-biological sources, such as CO_2 in the atmosphere, or organic carbon sources, such as carbohydrates. The organisms that can use a non-biological carbon source are known as autotrophs, which literally means *self-feeding*. The organisms that require an organic carbon source are the same that require an organic chemical energy source and are called heterotrophs. This information is summarised in **Figure 2**.

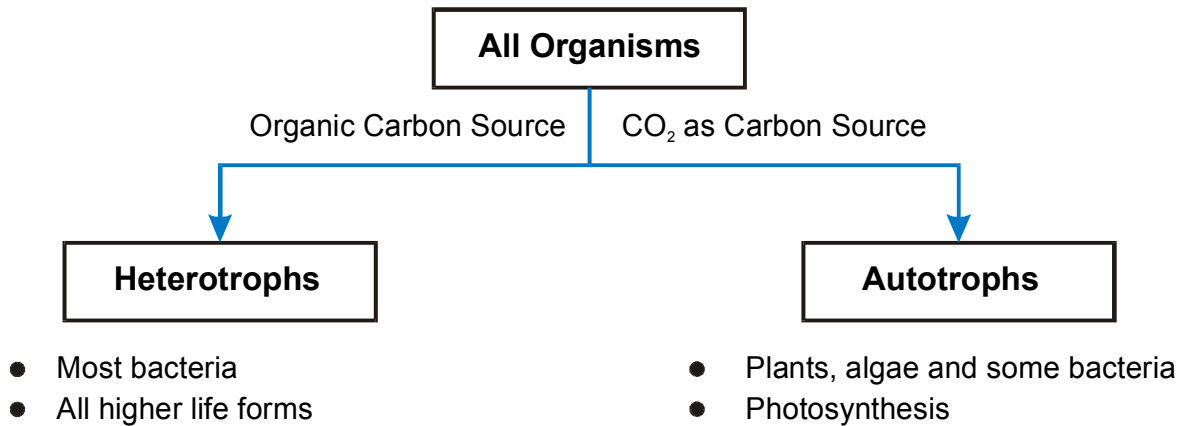


Figure 2. The characteristics of Heterotrophs and Autotrophs.

4.1.1.1 The Carbon Cycle.

The dependency of heterotrophs on carbon fixation by autotrophs is obvious. However, it is interesting to note that autotrophs are similarly dependent on heterotrophs for continual liberation of carbon dioxide. In fact, it has been estimated that autotrophs would deplete the biosphere of carbon dioxide within 300 years if the carbon cycle did not exist. Illustrations of the Carbon Cycle are shown below and overleaf in **Figures 3, 4 and 5**.

Another factor, highlighted by **Figures 3 and 5** is the liberation of oxygen as a result of photosynthesis which is of course essential for aerobic metabolism in which oxygen acts as the terminal electron acceptor, liberating large amounts of energy from breakdown of organic compounds.

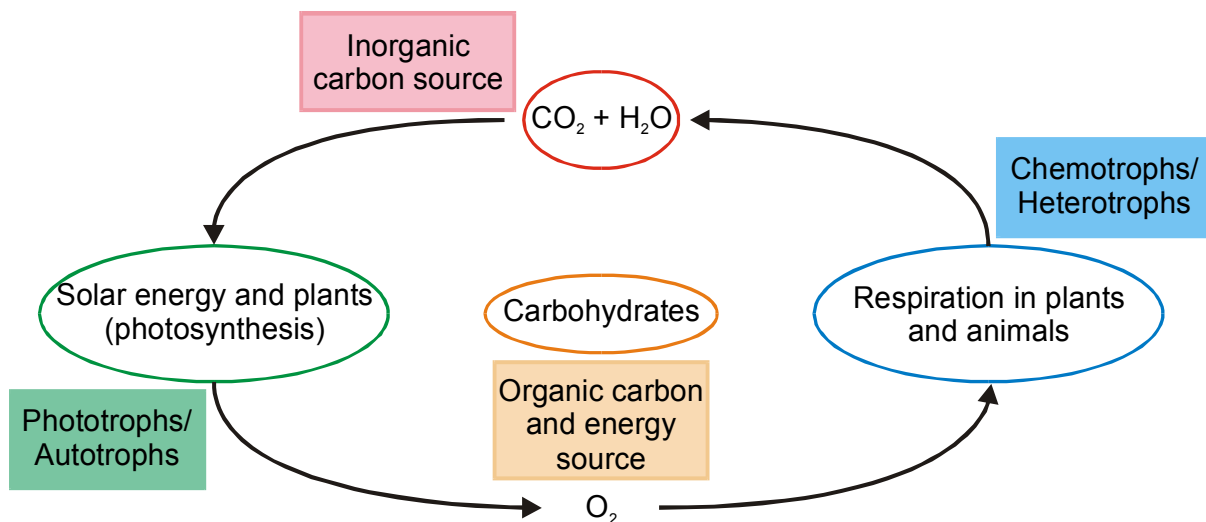


Figure 3. The Carbon Cycle.

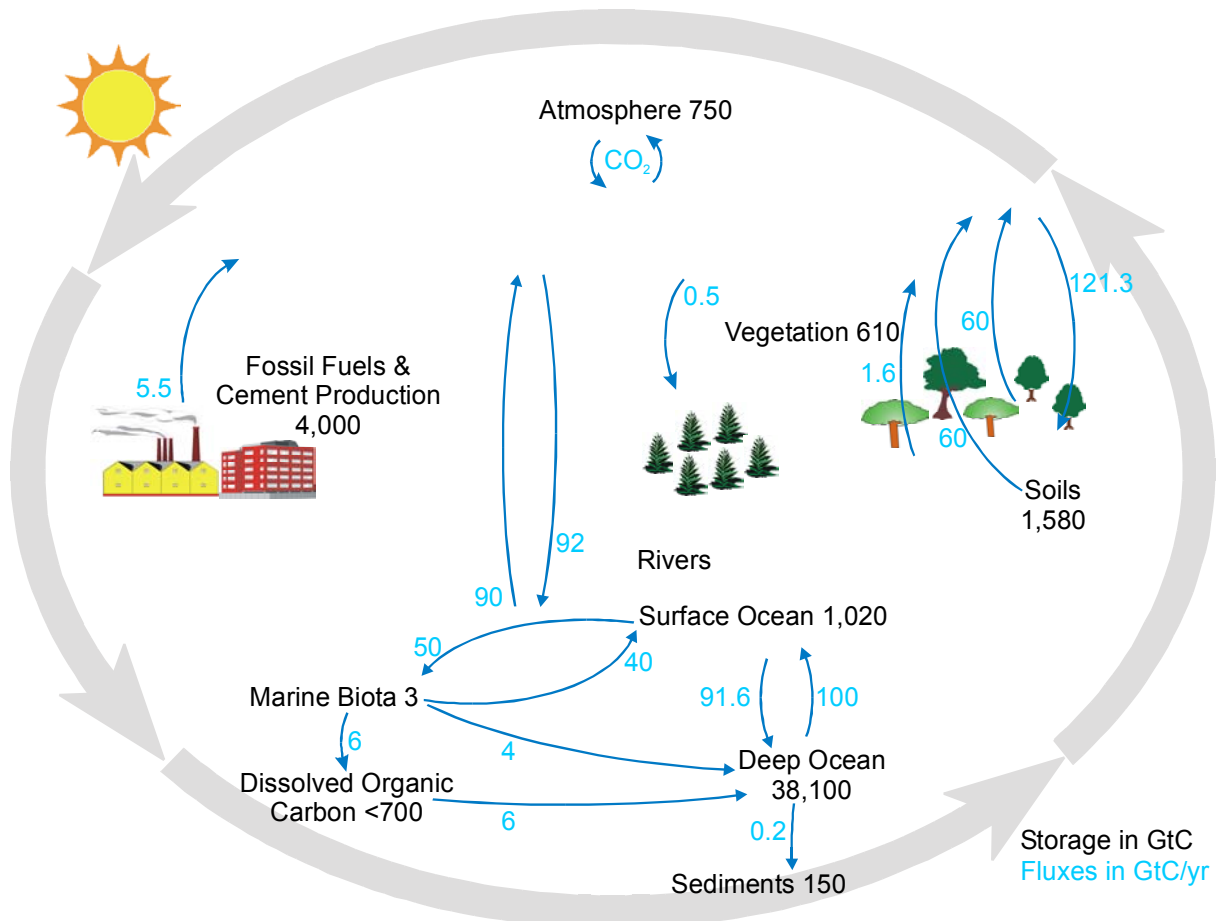


Figure 4. A more detailed diagram of the Carbon Cycle.

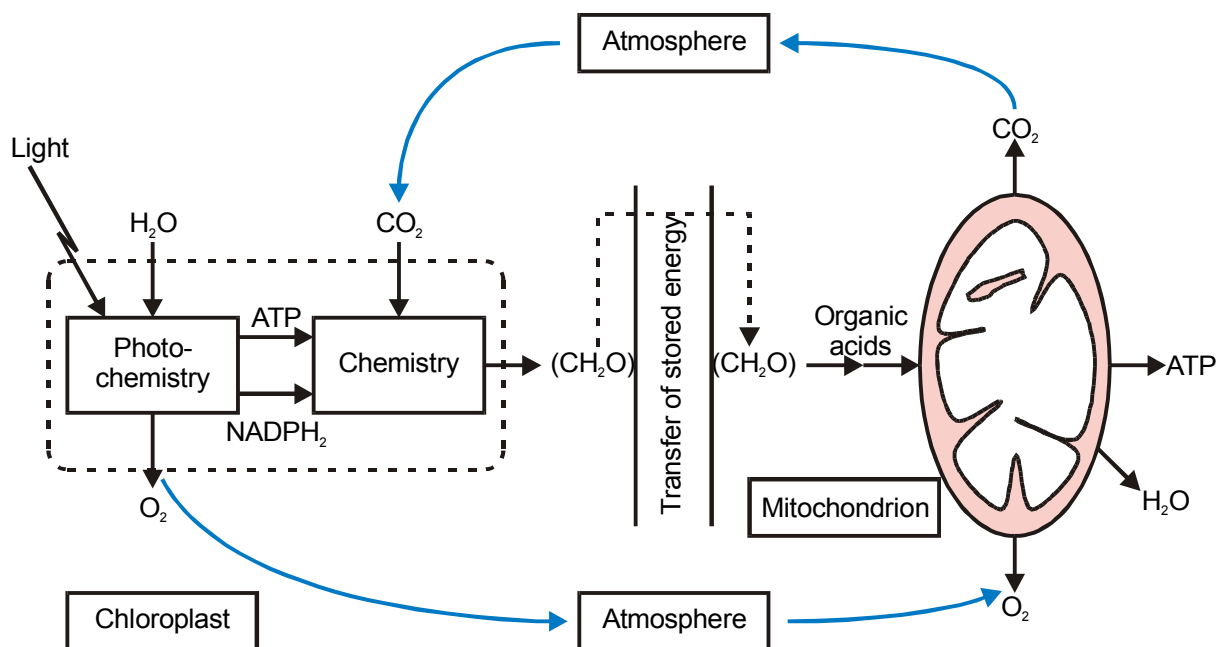


Figure 5. The CO₂ and O₂ cycle in the atmosphere and the cell.

The concepts that have just been looked at are shown in more detail in **Figure 6** overleaf.

Everything above the cell membrane is representative of photosynthesis.

The energy source is solar, which is used to convert inorganic compounds, such as water and carbon dioxide, to organic compounds that can be utilised by heterotrophic organisms for energy and carbon. This is done via glycolysis to produce pyruvate and energy in the form of ATP.

Aerobic metabolism uses the Krebs cycle to 36 ATP molecules, alternatively, anaerobic catabolism results in the formation of alcohol, acids and methane.

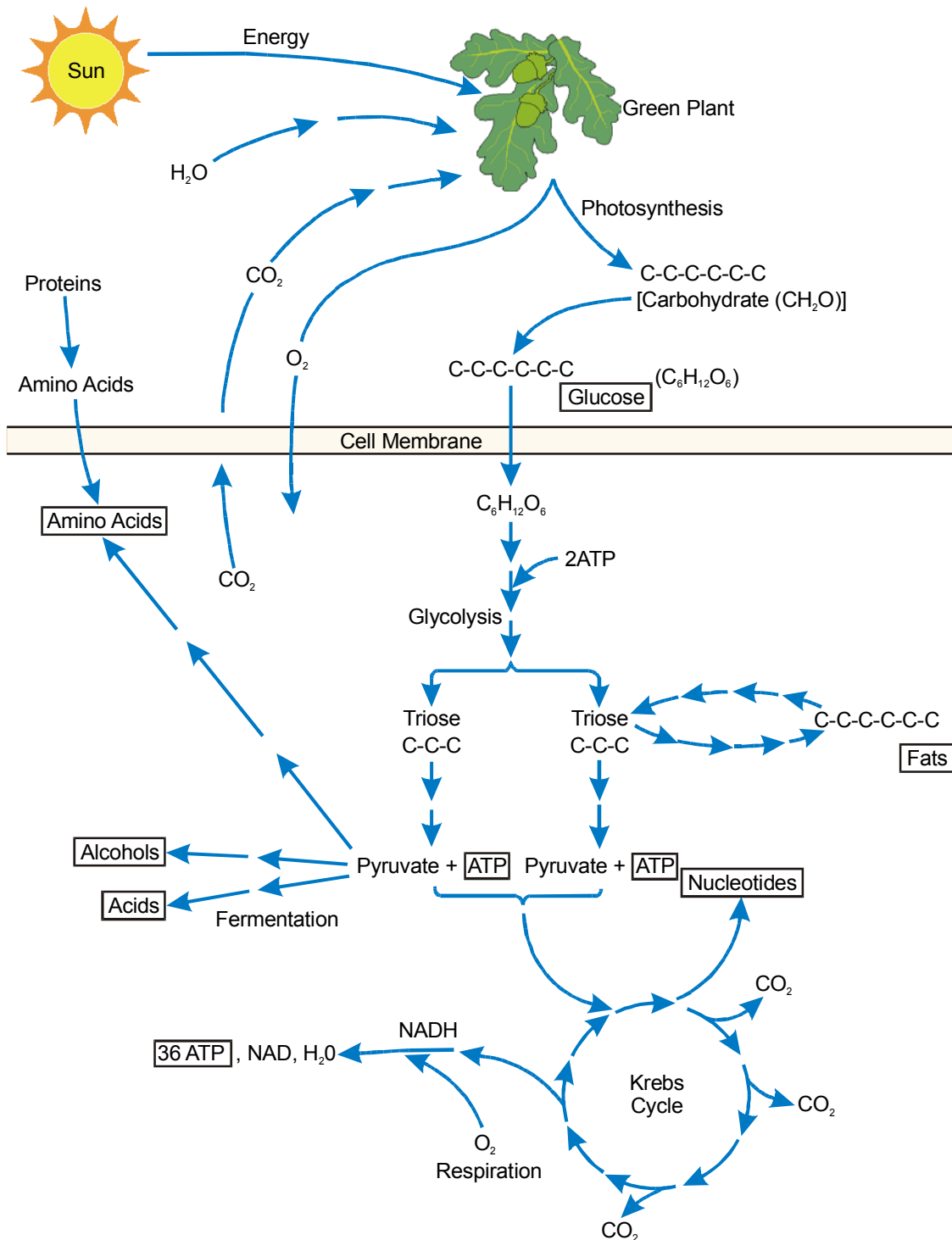
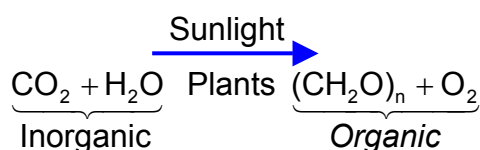


Figure 6. A simplified schematic diagram illustrating the source of cellular energy and the biosynthesis of cellular materials.

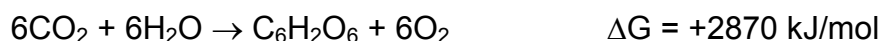
4.1.2 What is Photosynthesis?

The term photosynthesis literally means building up or assembly by light, which refers to the use of light as an energy source.

It is the process whereby plants, algae and some bacteria synthesise organic compounds from inorganic raw materials. This is an anabolic process and anabolic processes always require energy, in this case the energy is supplied from the sun. Because of this photosynthesis can also be described as the conversion of solar energy to chemical energy in the form of ATP. Photosynthesis is an oxidation – reduction process (see Section 2.2.3 of Unit 2).



During photosynthesis, carbon dioxide and water are converted to carbohydrates and oxygen in the presence of sunlight. We can illustrate this with the example of glucose synthesis via photosynthesis:



The positive ΔG tells us that a large amount of energy is required for this process, which is supplied by light energy. Thus, energy poor compounds, carbon dioxide and water are converted to energy rich compounds such as glucose.

4.1.2.1 Basic Outline of Photosynthesis

Now that we know what photosynthesis is we are going to look in more detail at all the individual steps required to convert carbon dioxide and water to complex carbohydrates. These steps are illustrated overleaf in **Figure 7**. As already mentioned, the energy source for photosynthesis is solar, which is captured by light sensitive pigments. Where and how this happens is the next stage of investigation.

Once the energy source has been dealt with, the process mechanisms of photosynthesis will be investigated in more detail. These are divided into two distinct reactions, the light reactions, which occur in the sunlight, and the dark reactions.

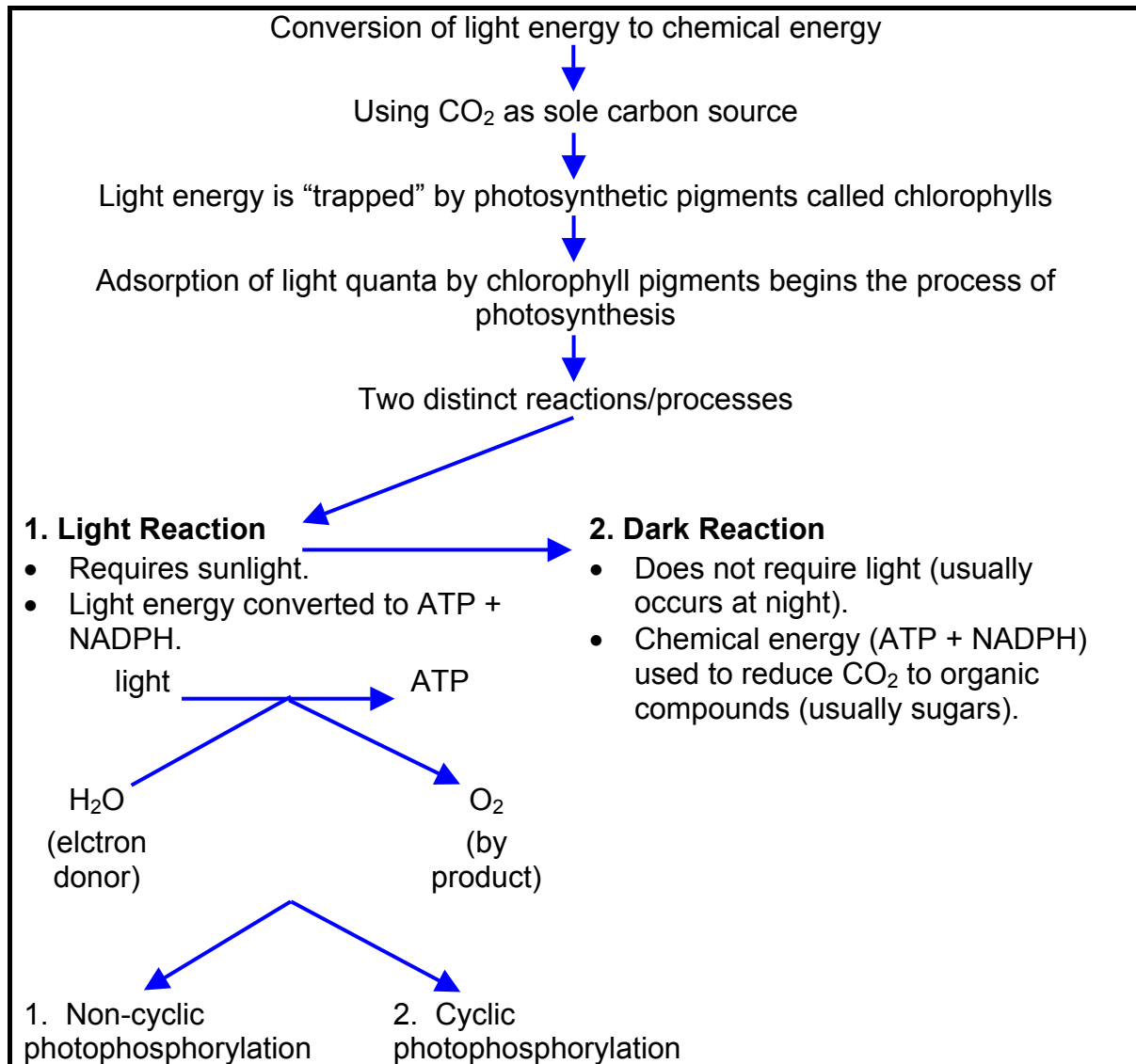


Figure 7. A simple outline of Photosynthesis.

4.1.3 Where does Photosynthesis occur in a Cell?

Firstly, photosynthesis occurs only in organisms that possess some type of **chlorophyll**, which is a green pigmented compound. The role of chlorophyll in light capture will be discussed later, for the meantime we are interested in where all this is located in the cell.

The chlorophyll pigments and all other components of the light-gathering apparatus are associated with special membrane systems, the photosynthetic membranes.

Bacteria are prokaryotic cells, and plants and algae are eukaryotic cells. The cytoplasm of prokaryotic and eukaryotic cells is arranged differently, with that of the eukaryotes being arranged into distinct organelles and that of the prokaryotes being more of an open-plan system. Therefore, the exact location of the photosynthetic apparatus depends on the type of cell. Eukaryotic cells have special **organelles** for photosynthesis known as **chloroplasts** (see **Figure 8** overleaf).

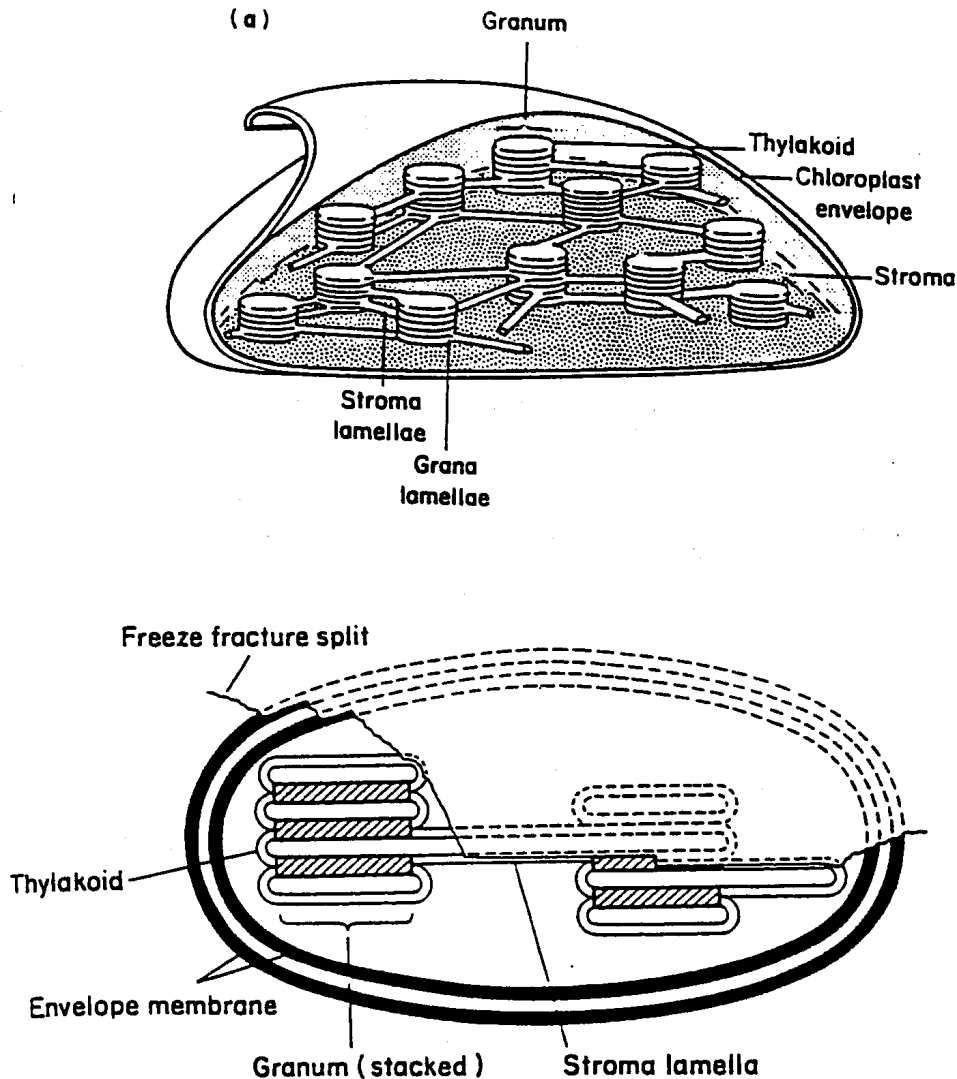


Figure 8. Cut-away representation of a chloroplast showing three-dimensional internal structure and a diagram of chloroplast structure as revealed by freeze etching in the electron microscope.

The chlorophyll pigments are attached to sheet-like lamellar membrane structures of the chloroplast, which are called **thylakoids**. Stacks of these thylakoids are collectively known as **grana**. The thylakoids are arranged so that the chloroplast is divided into two regions, the **matrix space**, which surrounds the thylakoids, and the **inner space** within the thylakoid array. This arrangement is important because it allows development of a light-driven pH gradient and proton-motive force, which can be used to synthesise ATP.

Proton motive force is the energised state of a membrane created by expulsion of protons through action of an electron transport chain.

Within the thylakoid membrane, the chlorophyll molecules are associated in complexes consisting of 200 to 300 molecules. Most of these chlorophyll molecules are light-harvesting or antenna molecules. These molecules are bound to proteins, which precisely control their orientation so that energy absorbed by one chlorophyll molecule can be efficiently transferred to another.

In this way, the harvested light is transferred until it reaches reaction-centre chlorophyll molecules in which light energy is converted to ATP. The significance of this antenna-like arrangement is that it allows a dramatic increase in the rate at which photosynthesis is carried out by permitting collection of light energy at a more rapid rate.

In prokaryotic cells, there are no chloroplasts and the chlorophyll molecules are incorporated into the cell membrane.

4.1.4 How is Light Energy captured by Chlorophyll?

Photosynthesis is driven by electromagnetic radiation in the range of 400 to 700 nm's, i.e. the region of visible light. This is known as Photosynthetically Active Radiation (PAR) and comprises 40 to 45% of the total light energy at ground level. Chlorophyll molecules typically adsorb radiation in the blue (455 to 500 nm) and red (620 to 775 nm) regions of the visible spectrum and reflects light in the green region, which explains why plants are green (see **Figure 9** below).

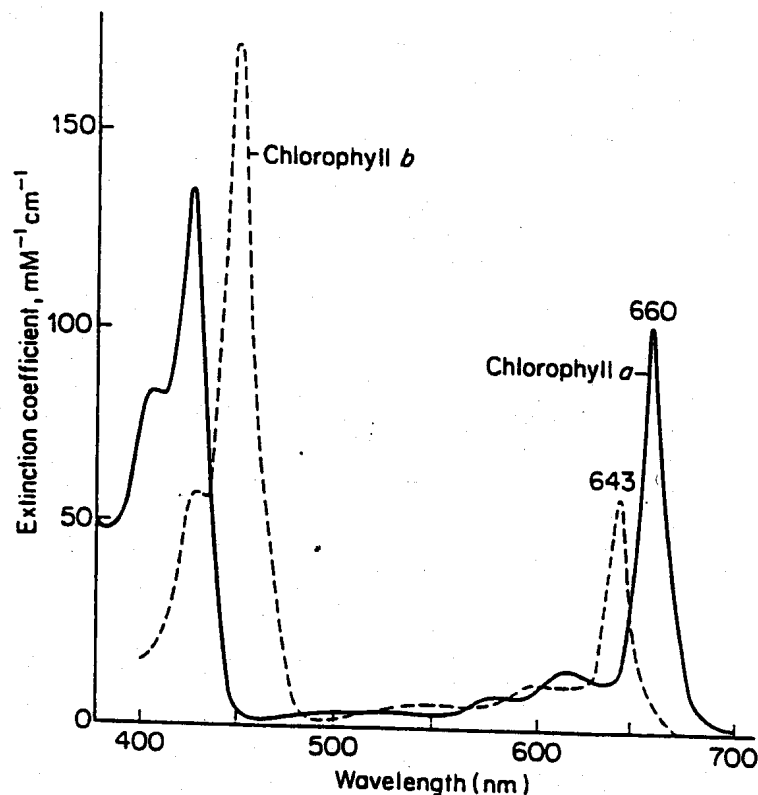


Figure 9. Absorption spectra of chlorophylls extracted in ether.

Each wavelength of light contains a precise amount of energy (a quantum of energy), which is carried by a photon. The energy of a photon is inversely proportional to the wavelength. For photosynthesis to occur, chlorophyll pigments must absorb the energy of a photon at a specific wavelength. That is, each type of chlorophyll molecule requires a specific wavelength of light in order to become activated or excited.

4.1.4.1 The Excited Atom!

An atom consists of a nucleus, which is formed of neutrons and protons. Around the nucleus are a number of orbitals in which electrons are found. In a normal state, the electrons occupy the orbitals that are closest to the nucleus, which gives the most stable arrangement (see **Figure 10** below).

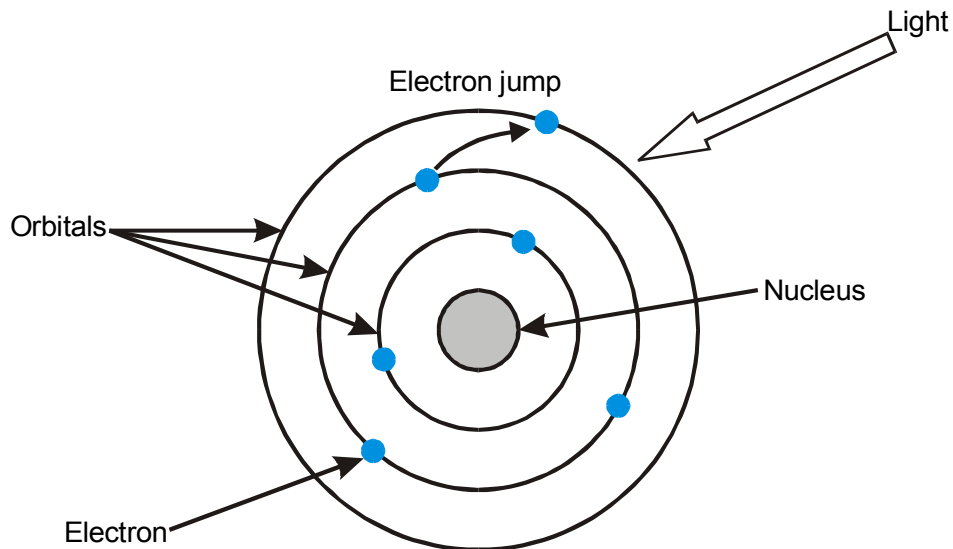


Figure 10. Schematic representation of the Atom.

For an electron to be moved to an orbital further away from the nucleus, a precise amount of energy must be supplied. Therefore, the electronic absorption of radiation can only take place if the impinging photon has an amount of energy equal to the difference of energy between the two orbitals. This will allow the electron to jump to the next orbital and excite the atom. In this way, the chlorophyll molecules capture light energy.

4.1.4.2 How is the Light Energy absorbed by Chlorophyll converted to Chemical Energy?

If pure chlorophyll is extracted from leaves and put into a test tube the molecules attain an excited state. But, as excited atoms are very unstable, they quickly return to the stable state, losing the absorbed energy as heat and fluorescence. However, with intact plant cells, the excited high energy electrons leave the chlorophyll molecules entirely and are led away from it by a chain of electron carrier enzymes. In this way generating chemical energy in the form of ATP by oxidation-reduction reactions.

The generation of ATP occurs in what is known as the “light reactions”, which can again be divided into two distinct kinds of pathways:

1. Non-cyclic photophosphorylation.
2. Cyclic photophosphorylation.

4.1.4.3 What is Photophosphorylation?

Photophosphorylation literally means addition of phosphorus by means of light. During these light reactions, hydrogen atoms are removed from water molecules and used to reduce NADP⁺, resulting in the by-product of oxygen. Simultaneously, ADP is phosphorylated to the high energy form, ATP. The structure of ATP is shown below in **Figure 11**.

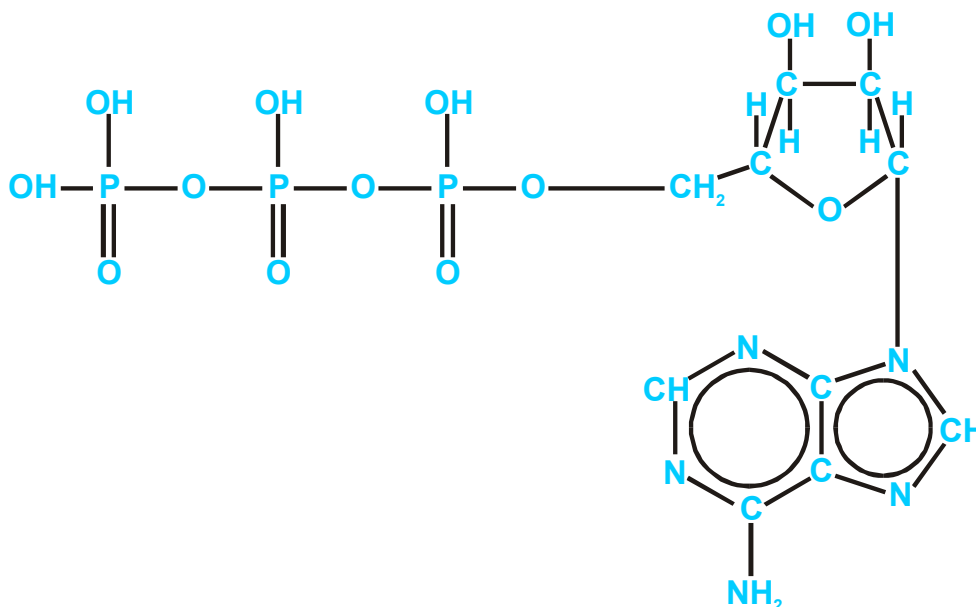
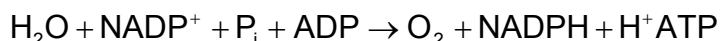


Figure 11. The structure of ATP.

This can be written down as:



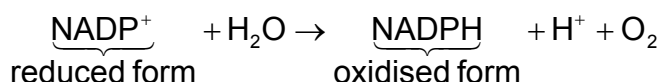
The synthesis of ATP has already been covered in Section 2.2.6 of Unit 2. To briefly recap, the ATP molecule is formed by addition of phosphate groups. The addition of each phosphate group requires a large input of energy, which is then stored in the ATP molecule and released when required by cleaving of the phosphate group.

It is also important to understand the role of Nicotinamide Adenine Di-nucleotide Phosphate (NADP) in this sequence.

The concept of the transfer of electrons in an oxidation-reduction reaction from an electron donor (in this case light) to an electron acceptor was introduced in Section 2.2.3 of Unit 2. The net energy change of the reaction sequence is determined by the difference in reduction potential between the primary donor and terminal acceptor. The transfer of electrons between the two involves intermediate carriers, which are constantly reduced and re-oxidised. NADP is a hydrogen atom carrier and always transfers two hydrogen atoms to the next carrier in the chain.

Oxidation has been defined in Section 2.2.3 of Unit 2 as loss of electrons (OIL) and reduction as gain of electrons (RIG), which is a strictly chemical definition. However, in biochemistry, oxidations and reductions frequently involve the transfer of whole hydrogen atoms instead of electrons. A hydrogen atom consists of a proton + electron so, when the electron is removed, the hydrogen atom is released as a hydrogen proton. Therefore, in the light reaction of photosynthesis, NADP⁺ is reduced by the addition of two hydrogen atoms and exists in the form NADPH + H⁺, although this is written as NADPH for the sake of simplicity. NADPH is a good electron donor, in other words, a good energy source.

The hydrogen donor required to reduce NADP⁺ comes from water in the case of green plants and algae:



In this case, oxygen is liberated as a by-product and the light reaction is known as non-cyclic photophosphorylation. Some photosynthetic bacteria can also use inorganic compounds such as hydrogen sulphide as an electron.

4.1.4.4 Non-cyclic Photophosphorylation

Light is used to generate both ATP (a high-energy compound) and NADPH (an excellent electron donor). The hydrogen ions for the latter coming from the splitting of water into oxygen (actually a by-product and of no use to the plant) and electrons. This process is carried out in plants and algae.

The path of electron flow in non-cyclic photophosphorylation resembles the letter Z turned on its side and, therefore, is sometimes referred to as the “Z-scheme” (see **Figure 12** overleaf).

In non-cyclic photophosphorylation, there are two photosystems involved in light-gathering, each having a distinct form of reaction centre. Photosystem I (shown as P700 in **Figure 12**) contains chlorophyll that adsorbs light at the wavelength of 700 nm (far red light) and Photosystem II (shown as P680 in **Figure 12**) adsorbs light best at shorter wavelengths (near red light).

1. Non-cyclic photophosphorylation begins with the absorption of light energy by P680, which converts it into a strong electron donor with a more negative reduction potential than the ‘base level’ P680.
2. The **excited electrons** are released from P680 (i.e. it is oxidised) and cascaded down a series of electron carriers in a thermodynamically favourable direction (negative to positive reduction potential). This generates a proton gradient from which ATP is produced.
3. The final electron acceptor for these electrons is P700, which also absorbs light energy and releases excited electrons that are used in the formation of a high energy end-product of photophosphorylation, NADPH (known as ‘reducing power’).

4. In order to return to its usual state, P680 must gain two electrons (i.e. be reduced) to compensate for the two electrons lost. These electrons are gained from the **splitting** of a **water** molecule. Oxygen is released as a by-product and the hydrogen atoms are split into electrons, which are used to reduce P680, and protons which are used to form the high-energy **NADPH**.

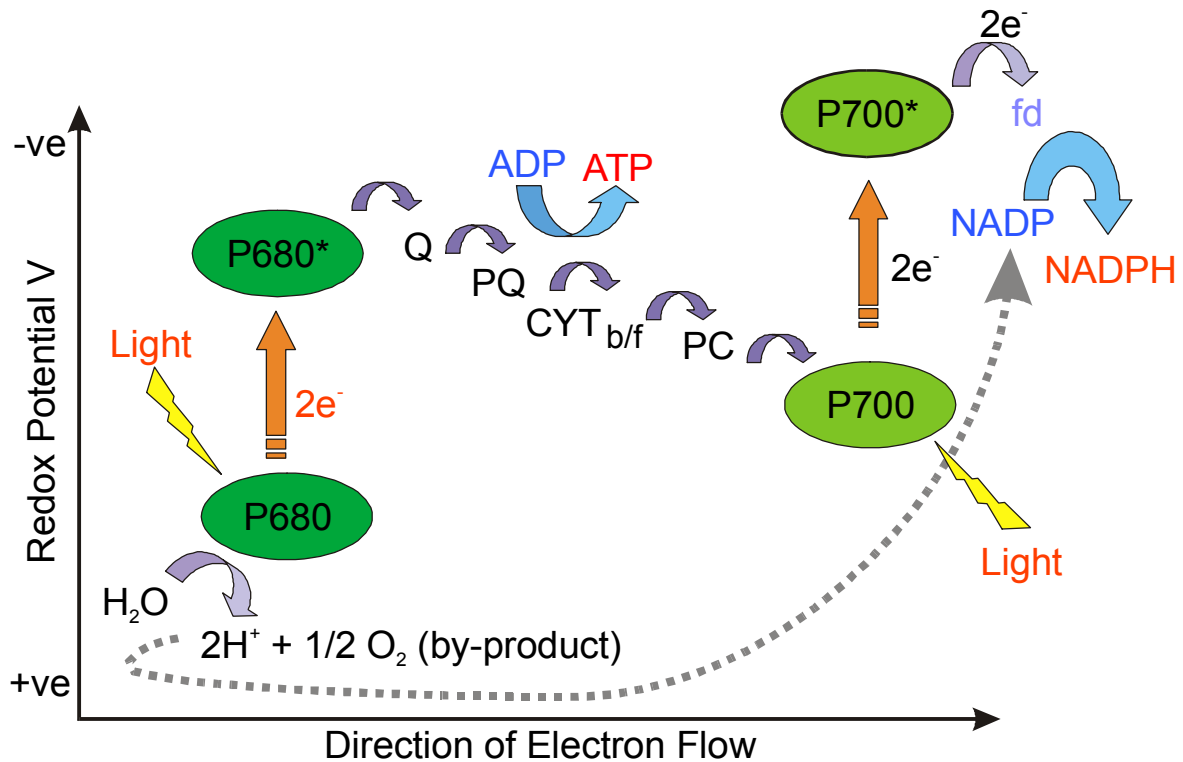


Figure 12. Illustration of Non-cyclic Photophosphorylation.

The essential feature of photosynthetic energy generation is the ability of the excited form of P680 and P700 to act as reducing agents (electron donors) at much lower redox potentials than when in the ground state.

4.1.4.5 Cyclic Photophosphorylation

This is an alternate pathway for ATP generation, used by some photosynthetic bacteria. The main feature of this pathway (see **Figure 13** overleaf) is that it is closed, i.e. there is no net input or consumption of electrons. Water is not required as a hydrogen and electron donor, consequently, there is no release of oxygen as a by-product, and no production of NADPH.

Only one photosystem, P700, is involved in cyclic photophosphorylation. Light is absorbed, exciting an electron, which is released and cascaded down a series of electron acceptors in a thermodynamically favourable direction, releasing energy for phosphorylation of ADP. The electron is returned to the oxidised P700, which is then reduced and can absorb light etc.

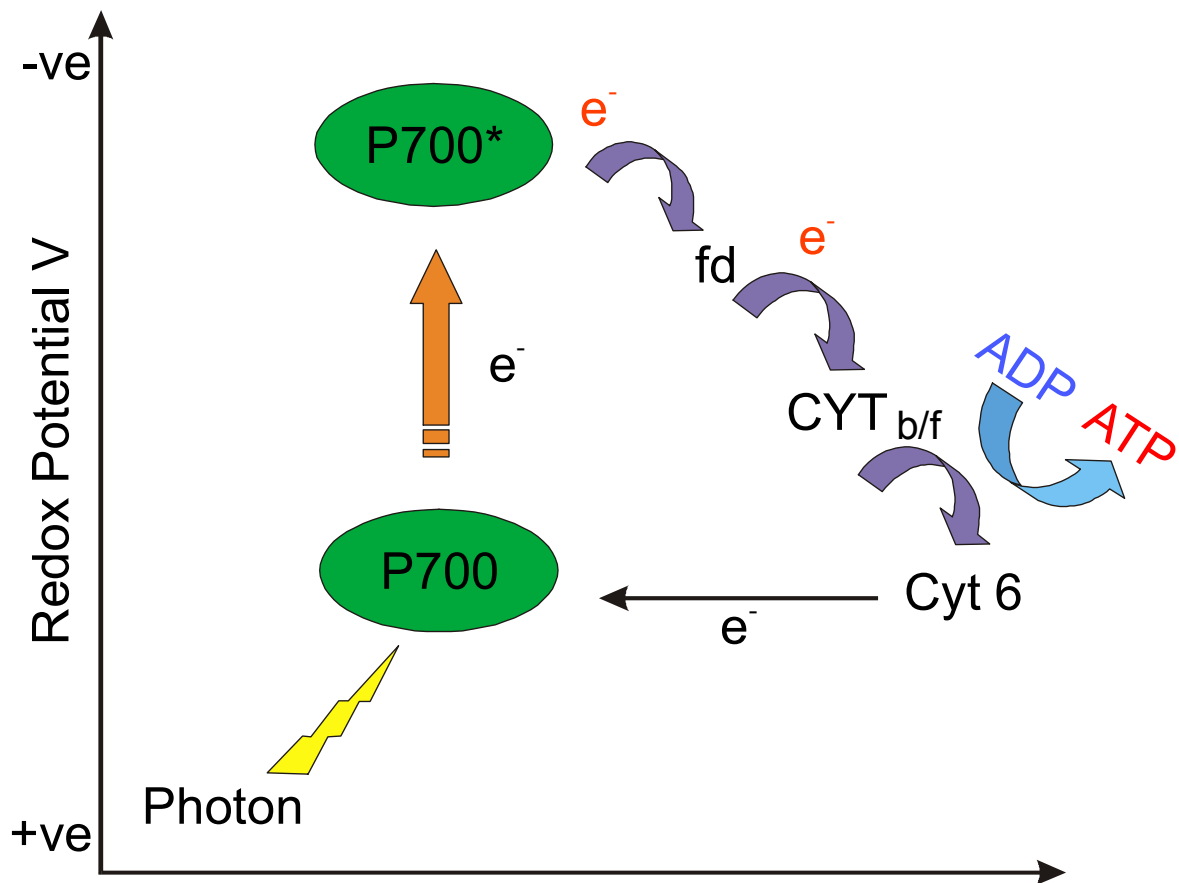


Figure 13. Illustration of Cyclic Photophosphorylation.

4.1.5 The Dark Reactions - Calvin Cycle

These are the reactions in which CO_2 is converted to organic matter. Autotrophs obtain all their carbon from CO_2 by a process known as CO_2 fixation, the reactions of which can occur in complete darkness, using ATP and reducing power (NADPH) generated during the light reactions of photosynthesis.

The pathway for CO_2 fixation is known as the **Calvin cycle** (see **Figure 14** overleaf).

This cycle is an anabolic pathway (i.e. synthesis of compounds) and, therefore, is reductive (requires input of electrons) and energy demanding. Reducing equivalents are obtained from NADPH and energy is obtained from ATP. CO_2 is reduced to the oxidation state of cell material.

1. The first step of the pathway is activation of a five carbon sugar molecule, Ribulose 5-phosphate to the reactive state in which it can accept CO_2 molecules. (The '5' refers to the carbon atom to which the phosphate is attached, it does not mean that there are five phosphate molecules). This state is Ribulose 1,5 bisphosphate and requires the input of six ATP molecules.

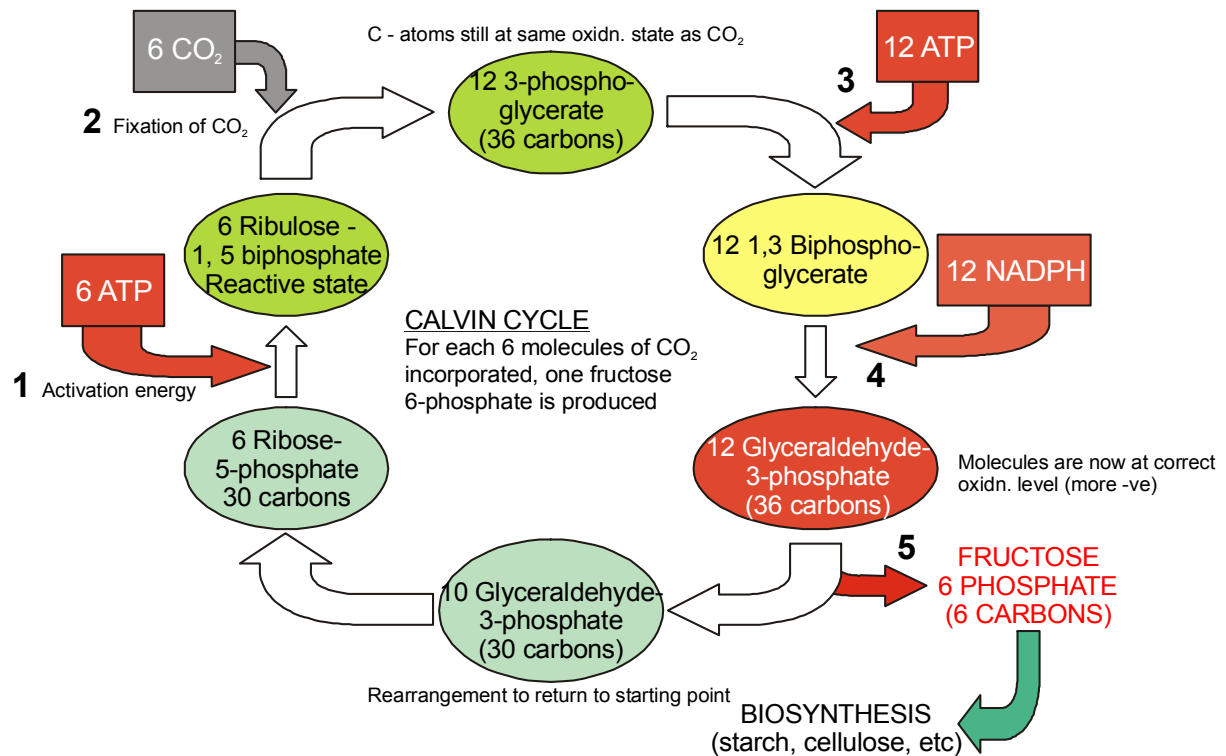


Figure 14. Diagrammatic representation of the Calvin Cycle.

- At this point six CO₂ molecules are fixed by incorporation into ribulose 1,5 bisphosphate. This temporarily forms an unstable intermediate compound, which splits to form 12 molecules of phosphoglyceric acid (PGA) (3C). These molecules contain the carbon atoms from CO₂ (carbon fixation). At this point the carbon atom in PGA is still at the same oxidation level it was in carbon dioxide and the next steps involve reduction of PGA to the oxidation level of carbohydrate.
- Reduction of PGA involves both input of energy and electrons, both of which are supplied by ATP and NADPH generated during light reactions of photosynthesis.
- From these steps 12 glyceraldehyde 3-phosphate molecules are generated, which are at the correct oxidation level. This is split into one molecule of fructose 6-phosphate (the sugar you find in fruit with an extra phosphate group to make it reactive for further biosynthesis into storage carbohydrates such as cellulose, starch) and 10 molecules of glyceraldehyde 3-phosphate.
- The next step involves rearrangement of the carbon atoms to yield six Ribulose-5 phosphate, which is the starting point of the reaction.

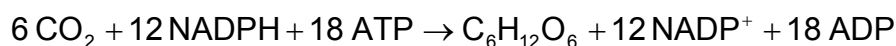
So, the important points of this reaction are:

- CO₂ is incorporated into a phosphorylated sugar molecule to form PGA.
- At this point it is still at the oxidation level of CO₂. That is, not a good electron donor.

- Input of reducing power and energy (light reaction) result in formation of a phosphorylated sugar (fructose), which is the energy-rich product of the Calvin cycle. That is, the energy-poor CO₂ has been converted to the energy-rich sugar with input of light energy in the form of ATP and NADPH.
- The glyceraldehyde phosphate compounds are rearranged to form the starting molecule ribulose 5-phosphate and the cycle continues.

4.1.6 The Energetics of CO₂ Fixation

The overall stoichiometry of CO₂ fixation is:

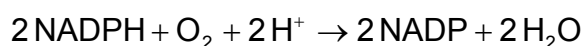


The $\Delta G = + 480 \text{ kJ/mol}$

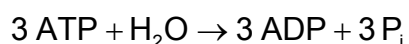
This means that 480,000 joules of energy are required to fix one mole of carbon dioxide to the level of glucose. The positive sign for the reaction indicates that the reaction is endergonic, i.e. a large amount of energy must be added to the system for the reaction to proceed. This energy is derived from the light phase of photosynthesis with the production of NADPH and ATP.

Two molecules of NADPH and three molecules of ATP are needed to fix one molecule of CO₂.

The energy present in NADPH and ATP is represented as follows:



$\Delta G = -440 \text{ kJ/mol}$.



$\Delta G = -92 \text{ kJ/mol}$.

The negative signs show that both reactions are exergonic, i.e. they produce usable energy, together providing 532 kJ/mol of usable energy. This energy is sufficient to reduce one CO₂ molecule to the level of glucose with about 50 kJ/mol to spare.

Looking at the above equations we can see that most of the energy is supplied by NADPH as reducing power, therefore, we can say that CO₂ fixation by photosynthesis is largely a reductive process.

$$440/532 \times 100 \approx 83\%$$

With respect to reduction potential:

The redox potential of sugars = -0.43 V (i.e. they are good electron donors, energy-rich).

Splitting of water provides the original source of reducing power and the redox potential of this couple is +0.82 V. Therefore, the overall change in redox potential is +0.82 to -0.43 V, which is 1.25 V. We can convert this into energy terms with the expression:

$$\Delta G = -nFE$$

$$\Delta G = 4 \times 9.64 \times 10^4 \times 1.25 = -482 \text{ kJ/mol}$$

Where: n = no of electrons (four electrons per molecule of O₂).

F = the Faraday (9.64 x 10⁴ J/volt equivalent).

E = difference in redox potential.

4.1.7 The Thermodynamic Efficiency of Photosynthesis

The actual thermodynamic efficiency of photosynthesis is not very high. If photosynthesis was 100% efficient in fixing CO₂, 528 kJ of photosynthetically active radiation would be required for every mole of CO₂ fixed. However, in reality, the light interception involves large energy losses in the form of:

- reflected light;
- dissipated heat; and
- photorespiration (reversal of the Calvin Cycle, i.e. sugar to carbon dioxide, which accounts for why plants also give off some Carbon dioxide).

This raises the requirements to 2600 - 3500 kJ PAR per mole of CO₂ fixed. If one considers the calorific value of sugar (468 kJ/mol), this represents an efficiency of 18%. However, maintenance and growth respiration of the plant utilises approximately 40% of the carbon fixed. Also, light saturation reduces efficiency by a further 30% in C₃ plants. Thus, the theoretical efficiency of photosynthesis in temperate crops is about 6.3%.

4.1.8 The Difference Between C₃ and C₄ Plants

The key enzyme in the Calvin Cycle is the one that catalyses the transformation of the 5-carbon sugar ribulose-1,5-biphosphate and the single-carbon carbon dioxide to form the 3-carbon compounds (3-phosphoglycerates) (Reaction step No. 2). This reaction has a very high delta-G of -12.4 kcal/mol. The enzyme is called ribulose-1-5-biphosphate carboxylase or **Rubisco**. Rubisco accounts for 16% of the protein content of the chloroplast and is believed to be the most abundant protein on Earth.

Rubisco is thought to be so abundant because of its inefficiencies. Rubisco will sometimes recognise oxygen as a substrate in the Calvin Cycle, instead of carbon dioxide, forming the compound phosphoglycolate, which is almost useless to the plant (see **Figure 15** overleaf). This reaction (the oxygenase reaction) directly competes with the regular reaction (the carboxylase reaction), at the same reaction site on the enzyme.

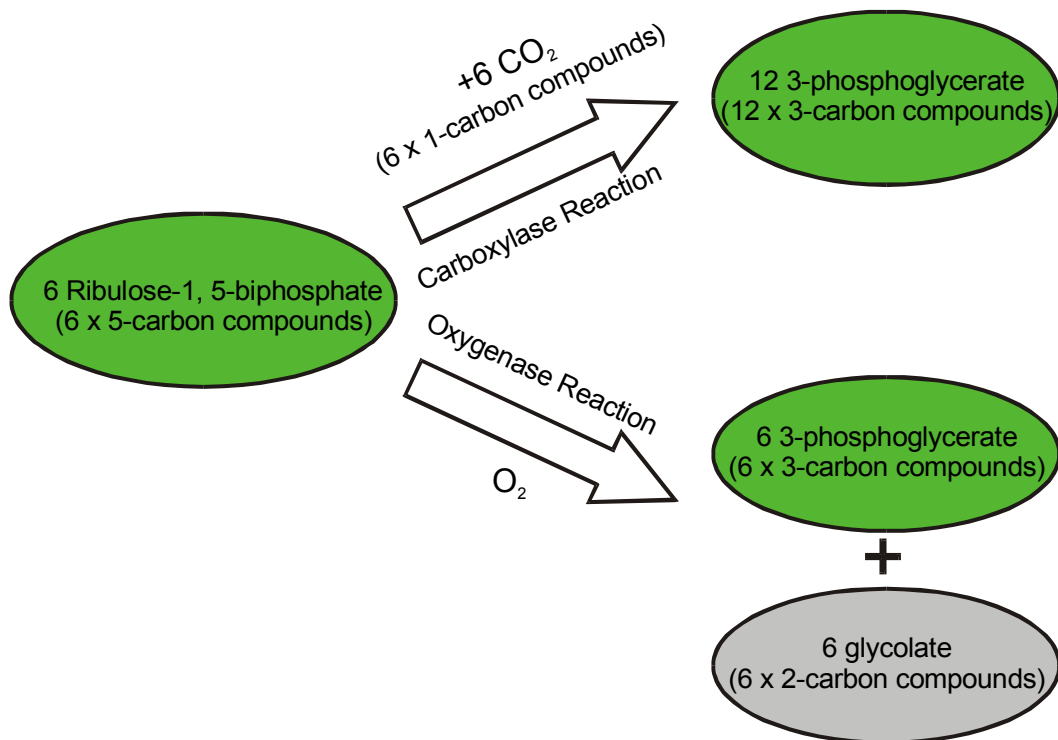


Figure 15. Competition between Oxygenase and Carboxylase reactions in the Calvin Cycle.

At 25 °C, the rate of the carboxylase reaction is four times that of the oxygenase reaction, so the plant is only about 20% inefficient. However, as the temperature rises, the balance in the air between oxygen and carbon dioxide changes and the carboxylase reaction is less and less dominant (i.e. photosynthesis is less and less efficient). Plants living in arid climates close the pores in their leaves to conserve moisture, which also gives the effect of creating a closed environment in which, as carbon dioxide gets used up in photosynthesis, the relative concentration of oxygen increases and the oxygenase reaction begins to dominate.

A solution has evolved to combat this problem. Plants living under hot and dry conditions have discovered a way to ensure a high carbon dioxide concentration in the immediate environment of Rubisco, thereby reducing the occurrence of the oxygenase reaction (see **Figure 16** overleaf).

This pathway is called the **C₄** pathway because it involves a **4-carbon intermediate in the outer cells**. The 4-carbon intermediate (malate) is split to form a 3-carbon molecule (pyruvate) and molecule of carbon dioxide. The carbon dioxide molecule is transported from the outer to the inner cell, thus, bringing a molecule of carbon dioxide right into the bundle sheath cells, next to the location of the Calvin Cycle. In this way, the plant ensures that the concentration of carbon dioxide at the site of Rubisco is high, so that the carboxylase and not the oxygenase reaction takes place.

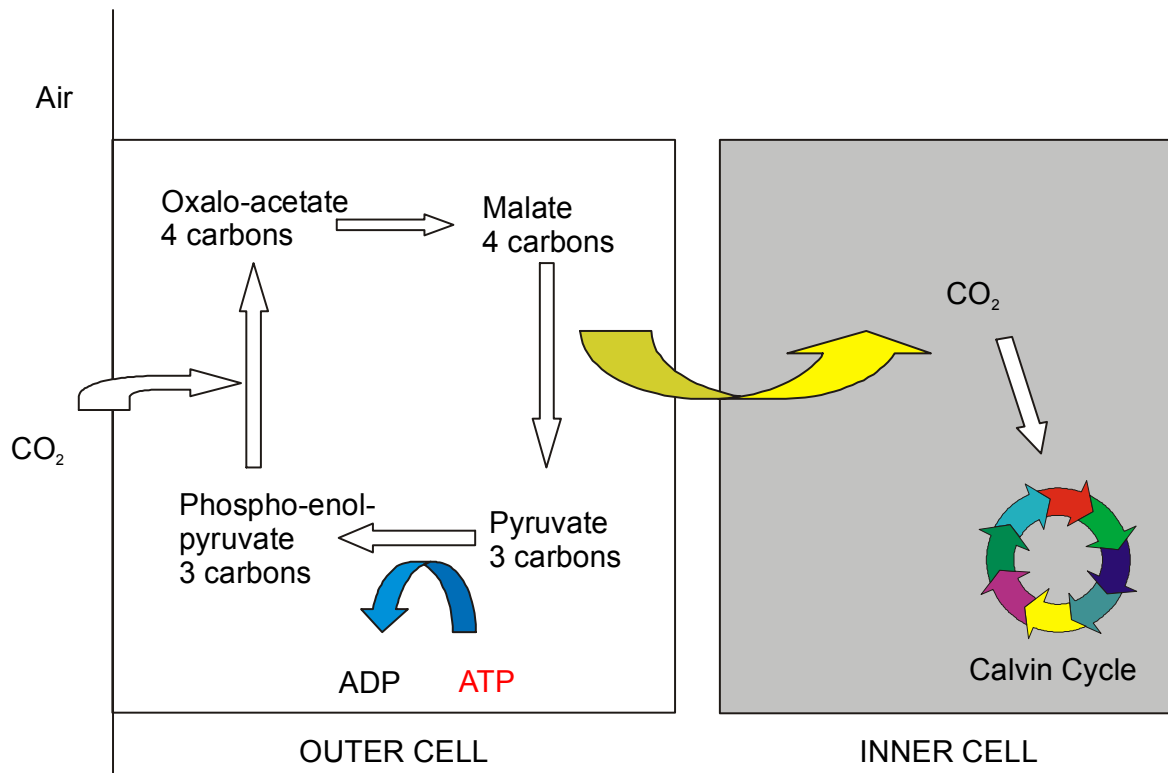


Figure 16. The C4 process, which ensures a constant supply of carbon dioxide at the site of the Calvin Cycle.

The conventional pathway of carbon fixation is called the **C3** pathway because it involves only the 3-carbon sugars.

NOTE. The C4 pathway still uses the conventional Calvin Cycle with its 3-carbon sugar intermediates; it just makes use of 4-carbon sugars to bring the carbon dioxide closer to the site of fixation.

So why don't all plants adopt the C4 process? Or, more correctly, why don't the C4 plants out-compete the C3 plants, which are inefficient?

You will note that it requires the input of one molecule of ATP to bring one molecule of carbon dioxide to the vicinity of the Rubisco enzyme. In moderate temperatures, the energy burden that this puts on the plant outweighs the advantage of eliminating the one in five times that Rubisco binds oxygen instead of carbon dioxide. In warmer climates, however, the C4 plants win with their novel strategy.



The following site is recommended for revision of photosynthesis:

<http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPS.html>

This site has been beautifully put together with really good diagrams and photographs and the information is correct and interesting.