Photosynthesis

**Syllabus**: The importance of photosynthesis in converting light energy to chemical energy.

Site of photosynthesis – the structure of dicotyledonous leaves in relation to photosynthesis; the structure of chloroplast as shown in electron micrographs. (refer to topic 'The cell --- organelles of cell'); the occurrence of different pigments in the chloroplast; the absorption spectra of chlorophyll pigments and the action spectrum of photosynthesis.

Photochemical reactions – an outline of the photochemical reactions:
1. electrons in chlorophylls are excited by light energy, without referring to photosystems I and II;
2. energy from these excited electrons generates ATP;
3. photolysis of water provides hydrogen for the reduction of NADP (nicotinamide adenine dinucleotide phosphate) and oxygen gas is released.

Carbon fixation – an outline of the Calvin cycle to show that:
1. carbon dioxide is accepted by a 5-C compound to form two molecules of a 3-C compound;
2. reduction of the 3-C compound by reduced NADP to triose phosphate, some of which combine to yield hexose phosphate which is subsequently metabolised to sucrose and starch;
3. metabolism of some of the triose phosphate to provide a continuous supply of the 5-C carbon dioxide acceptor.
   - triose phosphate can be used as a substrate to produce lipids and amino acids.

Factors affecting the rate of photosynthesis – the effects of light intensity, carbon dioxide concentration and temperature on the rate of photosynthesis; the concept of limiting factors, as exemplified by light intensity and carbon dioxide concentration; the principle for maximising plant growth in greenhouse by the control of light, temperature and carbon dioxide concentration.

**Photosynthesis (Photoautotrophic nutrition)**

Photosynthesis is the use of light energy to decompose water and to transfer hydrogen from it to carbon dioxide in the presence of photosynthetic pigment, i.e. chlorophyll. Through oxidation-reduction reactions, sugars is produced.

**A. Significance of photosynthesis**:
1. produce food for all the living organisms directly or indirectly
2. provide raw materials for building, or making other useful products, e.g. timber, furniture
3. balances the composition of atmospheric air by releasing oxygen and removing carbon dioxide from the air
4. formation of fossil fuels

**B. Site of photosynthesis**:
The major photosynthetic organ is the leaf. Its structure and function are closely related, i.e. the structure of the leaf is highly adapted to satisfy the process.

Leaf anatomy in relation to photosynthesis:
- a) leaves are thin, flattened and with large area to volume ratio, so as to have maximum exposure to sunlight
- b) chloroplast mostly found in palisade mesophyll in the upper layer of the leaves
- c) possessing stomata for gases exchange, mainly in the lower surface for dicotyledonous plant
- d) extensive system of intercellular space provide an internal atmosphere in contact with large number of cells
- e) the walls of the cells are saturated with water to dissolve and transport CO₂
- f) many small veins (with vascular bundles) bring a supply of water and minerals salts from roots in the xylem and carry away the food produced in the phloem
- g) a layer of transparent cuticle on upper and lower epidermis protects the leaf from desiccation and infection
Fig. 1. Structure of TS of a typical dicotyledonous leaf and showing the LS of a stoma.
Structure of dicotyledonous leaves:

a) Vascular tissue (supply water and remove photosynthetic products)
   - Water is carried through the xylem elements of the main vein in the midrib
   - Organic products of photosynthesis are carried to other parts of the plants through phloem elements (sieve tubes) in the veins

b) Stomata (for gaseous exchange)
   - The waxy cuticle covering the epidermis of leaves is permeable to CO₂ in some plants
   - But in most species, CO₂ reaches the mesophyll tissue mainly through the stomata
   - There are several patterns of stomatal distribution in leaves
     - In many mesophytic dorsoventral leaves, stomata are confined to the lower epidermis to reduce the risk of excess water loss
     - In the monocots bilateral leaves, about equal numbers of stomata occur on both surfaces
     - In the leaves of floating hydrophytes, the stomata are only found on the upper epidermis where it is exposed to air
     - In submerged leaves, no stomata is found, CO₂ diffuses directly
   - Each stoma consists of a pair of guard cells between which a pore is formed when the stoma is open
   - The stomata regulate the passage of CO₂, O₂ and water vapour across the surface of the leaf

c) Epidermis (allow light penetrate)
   - Very thin, i.e. light reaching the leaf surface has only to pass a short distance before reaching the mesophyll
   - Transparent, i.e. penetration of light is possible

d) Mesophyll
   - It is the tissue where the chloroplasts are mainly located
     [Note] Details of chloroplast refer to note “The Cell”.
   - There are two types of mesophyll
     1. Palisade mesophyll = just beneath the epidermis, packed closely, chloroplast densely located.
     2. Spongy mesophyll = located under the palisade Mesophyll, packed loosely with less chloroplast but lot of air space.

e) Orientation and size
   - In general the leaves of many plants grow so that the leaf blade is usually at right angle to the sunlight
   - This is achieved by the strengthening tissue e.g. collenchyma cells and sclerenchyma fibres
   - Leaves which are shaded (shaded leaves) have a large surface area than those exposed to full sunlight (sun leaves)
   - Shade leaves are also thinner and have more chloroplasts because of this shade leaves make efficient use of the dim light they receive
Exercise:

(92 II 2a(ii))
For guard cell, explain how its structure is related to its function(s). [2 marks]

(96 II 2a)
Explain how the structural features of a dicotyledonous leaf make it an efficient organ for photosynthesis. [8 marks]

Photosynthetic pigments:
- the pigments are mainly for absorbing light energy, then converting it to chemical energy
- they are located on the chloroplast membranes (lamellae and intergranal lamellae)
- the pigments in the chloroplast are in the form of a mixture
- they can be separated by paper chromatography when the green pigment is extracted from leaves with acetone
- about five pigments can be identified by this method

a) Primary photosynthetic pigment e.g. chlorophyll a & b
   - chlorophyll are green because they reflect green light
   - two major components are identified: chlorophyll a & b
   - they absorb light from both red and blue/violet parts of the spectrum
   - chlorophyll a is the most abundant pigment and is universal occurrence in all photosynthesising plants
   - its function is to absorb light energy and convert it into chemical energy
   - the other pigments do this too and then probably hand on the energy to chlorophyll a

b) Accessory pigments e.g. carotenoids
   - absorb light energy from various regions of the light spectrum and then pass on to chlorophyll
   - allowing plants to use light of more different wavelengths
   - one common example is carotenoids
     - yellow, orange, red or brown pigments
     - absorb strongly in the blue and blue-green range
     - they are usually masked by the green chlorophyll but can be seen in leaves prior to leaf-fall since chlorophyll break down first
     - they may also protect chlorophyll from excess light

Light absorption:
- in general leaves absorb about 83% of light, while reflecting 12% and transmitting 5%
- of the 83% absorbed, only 4% is actually used by the plants during photosynthesis, the remainder is dissipated as heat

a) Absorption spectrum
   - it is a plot of the absorbance by a substance of radiation at different wavelengths
   - the absorption spectrum of a substance can give information about the identity or quantity of a substance
   - chlorophyll, for example, have absorption peaks in the red and blue, and therefore reflect green light
b) Action spectrum
- it shows the amount of wavelengths which have been absorbed by the leaves actually used in photosynthesis
- they can be shown by exposing leaves to different coloured lights and that estimating the amount of carbohydrate formed in each case

Exercise: (94 II 1a)
Outline the role of accessory pigment in photosynthesis. Name one group of accessory pigments and state its absorption spectrum. [3 marks]

C. Photochemical reactions:
- Photosynthesis involves two successive steps --- light reactions and dark reactions.
- The light reactions take place in the grana of the chloroplasts where chlorophyll can be found located on the membranes.
- The dark reactions take place at the stroma of the chloroplasts where is absent from chlorophyll.

Light reactions (light dependent reactions):
- Excitation of pigments by light
  - pigments are chemicals that absorb visible light
  - this causes the excitation of certain electrons to ‘excited states’ from ground state, that is the electrons absorb energy
  - the excited state is usually unstable and the molecules returns to its ‘ground state’ and releasing its energy
  - the excited pigments lose electrons leaving positive ‘holes’ in their molecules

\[
\begin{align*}
\text{Chlorophyll}^{\text{(reduced form)}} & \quad \text{light energy} \\
\text{Chlorophyll}^+ + e^- & \quad \text{Chlorophyll}^{\text{(oxidised form)}}
\end{align*}
\]
- each electron lost is accepted by another molecules, the so-called ‘electron acceptor’ (NADP)
- the chlorophyll is oxidised and the electron acceptor is reduced
- chlorophyll is described as an electron donor
b) Conversion of light energy
- light energy is absorbed by an assembly of pigment molecules (photosystems I & II), raising its energy level to an excited state
- the excitation energy is transferred to specialized pigment molecules which called the reaction centre, from which an electron is released to reduce NADP

c) The mechanisms
- the synthesis of ATP by the light-induced phosphorylation (addition of a phosphate group to a molecule) of ADP is known as photophosphorylation
- there are two types of photophosphorylation
  1. Non-cyclic photophosphorylation

\[
\text{H}_2\text{O} + \text{NADP} + 2\text{ADP} + \text{Pi} \xrightarrow{\text{light energy, chlorophyll}} \frac{1}{2} \text{O}_2 + \text{NADPH}_2 + 2\text{ATP}
\]
- the electrons are transported from water on a unidirectional manner and do not cycle back to the chlorophyll
- it is initiated by light shining on PSI and PSII
- in order to replace the loss of electron during excitation by light, following processes occurs
  - electrons emitted from PSII return to PSI after release their energy content to ADP
  - water is photolyzed to give electrons which flow to PSII via electron carrier Z and oxygen is produced as a by-product of photosynthesis
  - finally, electrons pass downhill from Y to NADP and combine with hydrogen ions (from photolysis of water) to form NADPH\(_2\)
- Importance of non-cyclic photophosphorylation
  1. by photolysis of water, it provides hydrogen ions for the reduction of NADP to NADPH\(_2\) which is essential in the carbon dioxide fixation in dark reaction
  2. by electron flow, solar energy is incorporated into chemical energy (ATP), this is known as photophosphorylation
  3. by producing ATP, the process provides chemical energy for the subsequent synthesis of carbohydrates in the dark reaction, no request of external ATP from mitochondrion
  4. oxygen is produced as the result of photolysis

2. Cyclic photophosphorylation
- when only PSI is activated, no electrons are removed from water (this can be illustrated by lack of O\(_2\) evolved)
- electrons from Y are recycled back to PSI via the chain of electron carriers
- their excitation energy is for ATP production
- there is no NADPH\(_2\) produced

3. Comparison of cyclic and non-cyclic photophosphorylation

<table>
<thead>
<tr>
<th>Pathway of electrons</th>
<th>Non-cyclic</th>
<th>Cyclic</th>
</tr>
</thead>
<tbody>
<tr>
<td>First e(^{-}) donor</td>
<td>water</td>
<td>P700 (PSI)</td>
</tr>
<tr>
<td>Last e(^{-}) acceptor</td>
<td>NADP</td>
<td>P700 (PSI)</td>
</tr>
<tr>
<td>Products</td>
<td>useful : ATP, NADPH(_2) waste : O(_2)</td>
<td>useful : ATP only</td>
</tr>
<tr>
<td>Photosystems involved</td>
<td>PSI and PSII</td>
<td>PSI only</td>
</tr>
</tbody>
</table>
Dark reaction (light independent reaction) :-
- the reduction of CO₂ to form carbohydrates is involved
- it is an energy (ATP) consuming process
- the reactions are controlled by enzymes and their sequence was determined by Calvin during the period 1946 - 1953, so the dark reaction cycle is also called Calvin cycle

a) Stages in Calvin cycle

1. Carbon dioxide Fixation
   - the CO₂ acceptor is a 5C compound
   - in the action of an enzyme, a temporary 6C compound is formed from the combination of CO₂ with 5C compound
   - later this unstable 6C sugar will split into two 3C compound

   \[ 5C \text{ compound} + CO₂ + H₂O \rightarrow 2 \text{ 3C compound} \]

2. Reduction phase
   - the 3C compound is the first stable product of photosynthesis
   - later it is reduced by NADPH₂ to form triose phosphate (TP), the energy required comes from ATP
   - the NADP and ADP then return to light reaction again to carry hydrogen ions and energy respectively

   \[ 3C \text{ compound} + ATP + NADPH₂ \rightarrow TP + NADP + ADP + Pi \]

3. Regeneration of the carbon dioxide acceptor, 5C compound
   - some of the TP has to be used to regenerate the 5C compound consumed in the first reaction
   - this process involves a complex cycle and ATP is used
1. Carbon dioxide diffuses into the leaf through the stomata and dissolves in the moisture on the walls of the palisade cells. It diffuses through the cell membrane, cytoplasm and chloroplast membrane into the stroma of the chloroplast.

2. The carbon dioxide combines with a 5-carbon compound called *ribulose bisphosphate* to form an unstable 6-carbon intermediate.

3. The 6-carbon intermediate breaks down into two molecules of the 3-carbon compound.

4. Some of the ATP produced during the light stage is used to convert 3C compound into *triose phosphate* (TP).

5. The reduced nicotinamide adenine dinucleotide phosphate (NADPH + H⁺) from the light reaction is necessary for the reduction of the 3C compound to TP. NADP⁺ is regenerated and this returns to the light stage to accept more hydrogen.

6. Pairs of triose phosphate molecules are combined to produce an intermediate hexose sugar.

7. The hexose sugar is polymerized to form starch which is stored by the plant.

8. Not all triose phosphate is combined to form starch. A portion of it is used to regenerate the original carbon dioxide acceptor, ribulose bisphosphate. Five molecules of the 3-carbon triose phosphate can regenerate three molecules of the 5-carbon ribulose bisphosphate. More of the ATP from the light reaction is needed to provide the energy for this conversion.

Fig. 4 Summary of dark reactions of photosynthesis.

Fig. 5 Summary of photosynthesis.
Exercise:
(96 II 2ab)
Illustrate the Calvin cycle (carbon fixation) using a flow diagram and state which intermediate is used for the synthesis of carbohydrates. Name ONE product of this synthesis.  

(90 I 3)
Explain why the following equation is an unsatisfactory summary of the process of photosynthesis:

\[
6 \text{CO}_2 + 6 \text{H}_2\text{O} \xrightarrow{\text{light}} \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2
\]

(94 II 1b)
Contrast the ‘light dependent reactions’ with the ‘light independent reactions’ in photosynthesis.

b) Importance of dark reaction:-
- the major function of dark reaction is to fix carbon dioxide to 3C compound from the air
- later the 3C compound undergoes various metabolic pathways for lipids, amino acids and complex sugar synthesis

1. Synthesis of carbohydrate
   - carbohydrates are synthesized in a process which is a reversal of glycolysis
   - the two common carbohydrate products are sucrose and starch
   - sucrose is the form in which carbohydrate is exported from the leaf in the phloem
   - starch is a storage product and is the most easily detected product of photosynthesis

   \[
   \text{3C compound} \xrightarrow{\text{reduction}} \text{TP} \xrightarrow{\text{condensation}} \text{Sucrose} \Rightarrow \text{transport out of leaf} \]

   \[
   \text{3C compound} \xrightarrow{\text{reduction}} \text{TP} \xrightarrow{\text{condensation}} \text{Sucrose} \Rightarrow \text{storage form}
   \]

2. Synthesis of lipids
   - 3C compound enters the glycolytic pathway and is combined with coenzyme A to form acetyl coenzyme A
   - this is converted to fatty acids in both cytoplasm and chloroplasts (not in mitochondria where breakdown of fatty acids occurs)
   - glycerol on the other hand is made from triose phosphate (TP)

3. Synthesis of amino acids
   - 3C compound and TP contain the elements carbon, hydrogen and oxygen
   - nitrogen, sulphur and phosphorus are also needed if amino acids and proteins are to be made
   - plants obtains these elements from the soil water
   - higher plants are able to synthesize all their amino acids using NH\(3\) or NO\(3^-\) as the nitrogen source and 3C compound from photosynthesis

   (i) \[
   \text{NO}_3^- \xrightarrow{\text{reduction, enzyme I}} \text{NO}_2^- \xrightarrow{\text{reduction, enzyme II}} \text{NH}_3
   \]

   (ii) \[
   \text{NH}_3 + \text{Kreb’ s cycle acids (i.e.}\alpha\text{-ketoglutaric acid)} \xrightarrow{\text{amination}} \text{amino acid}
   \]
the above two reactions are important for amino acids production, especially reaction (ii) which is the major route of entry of NH$_3$ into amino acids.

by the process called transamination, other amino acids can be made.

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**D. Factors affecting the rate of photosynthesis:**

a) Light intensity

- it affects the photochemical reactions (light reactions) so controlling the rate of production of ATP and NADPH$_2$
- the rate of photosynthesis is directly proportional to the light intensity up to the point of saturation
- however, very high light intensities may bleach chlorophyll and retard photosynthesis
- in natural condition, plants are usually protected by devices such as thick and waxy cuticle to against the strong light
- on bright sunny days the CO$_2$ concentration of the atmosphere is usually the limiting factor but in cloudy days, light may be the limiting factor
b) Carbon dioxide concentration
- CO$_2$ is needed in the dark reactions where it is fixed into organic 3C compound
- under normal field conditions, CO$_2$ is the major limiting factor in photosynthesis
- the short-term optimum is about 0.5%, but this can be damaging over long periods (by exerting a depressive effects on the plant’s respiration); then the optimum is about 0.1%
- this has led to some greenhouse crops, such as tomatoes, berries grown in CO$_2$ enriched atmosphere to achieve high yields in greenhouse

c) Temperature
- as in all life processes, photosynthesis is restricted to a temperature range that roughly corresponds to that tolerated by protein compounds which generally active at temperatures between 0 - 60 °C
- although the photochemical part of photosynthesis is independent of temperature, the biochemical part (dark reaction), which is controlled by enzyme activity, is strictly temperature-dependent
- in general, increase in temperature results in an increase of photosynthesis when all other factors are not limiting
  - the increase is linear at low temperature (from 0 °C)
  - the increase begins to drop as higher temperatures (about 40 °C) are reached
  - finally it reaches an optimum (about 60°C) above which photosynthesis is inhibited

Concept of limiting factors:

a) The three cardinal points
- there is a minimum, optimum and maximum point for each factor in relation to photosynthesis
- for example
  1. a minimum temperature below which no photosynthesis takes place
  2. an optimum temperature at which the highest rate takes place
  3. a maximum temperature above which no photosynthesis will take place
- it should be noted that when dealing with biological materials, extremes of any controlling factor would have a ‘detrimental effect’ (i.e. freezing, denaturation of protein etc.)
- on continual increase of the factor under observation, the rate of reaction (i.e. photosynthesis) diminishing until no longer measurable

b) Factors considered
- since the rate of a biochemical process which like photosynthesis, involves a series of reaction, it is impossible to treat the external factors individually
- they have to be treated in relation to one another

c) Blackman’s principle of limiting factors
- this principle states that when a chemical process is affected by more than one factors, its rate is limited by that factor which is nearest its minimum value
- it is that factor which directly affects a process if its quantity is changed
- this factor is limiting factor
for example
1. Temperature and CO₂
   - in the graphs below, experiment 1 - 4 show that once light intensity is no longer limiting (its value is tend to maximum), products (NADPH₂ & ATP) are largely produced
   - since the dark reactions are enzyme-controlled, an increase in temperature from 15 °C to 25 °C results in an increased rate of photosynthesis (compare experiments 2 & 1, or 4 & 3)

![Diagram of photosynthesis rate vs. light intensity](image)

2. Sunlight intensity
   - from the minimum point to point X, sunlight intensity acts as a limiting factor at 15 °C (experiment 2 & 4)
   - the graph within this range is a linear relationship between the light intensity and the rate of photosynthesis
   - it means that even increasing CO₂ concentration within this range will not affect much the photosynthetic rate since the products (NADPH₂ & ATP) from light reaction is insufficient as the low light intensity for use in the dark reaction
   - from the minimum point to point Y, sunlight intensity acts as limiting factor at the concentration of CO₂ is low (0.4%) (experiments 1 & 2), the reason is same as above

Exercise : (93 II 3b)
What is meant by ‘limiting factor’? With the aid of labelled curves, explain how and why carbon dioxide can be a limiting factor to photosynthesis. [8 marks]

E. Photosynthesis versus respiration:
The equation that has puzzled many investigators in the past and still remains a problem today is, can photosynthesis be called the reversed of respiration?

\[
\text{CO}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{photosynthesis}} [\text{CH}_2\text{O}]_6 + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{respiration}}
\]

The above equation is highly simplified, actually there are many difference between 2 processes
Table 1 Comparison of photosynthesis and Respiration.

<table>
<thead>
<tr>
<th></th>
<th>Photosynthesis</th>
<th>Respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cell / organelle involved</td>
<td>Plant cells with chloroplast</td>
<td>Mitochondria of all living cells</td>
</tr>
<tr>
<td>2. Light requirement</td>
<td>Essential</td>
<td>Not necessary</td>
</tr>
<tr>
<td>3. Raw materials</td>
<td>CO₂ and water</td>
<td>Food substances and oxygen</td>
</tr>
<tr>
<td>4. End product</td>
<td>Carbohydrate and oxygen</td>
<td>Water and carbon dioxide</td>
</tr>
<tr>
<td>5. Energy involved</td>
<td>Light energy is absorbed</td>
<td>Heat energy and ATP are released</td>
</tr>
<tr>
<td>6. ATP formation</td>
<td>ATP is formed by photophosphorylation</td>
<td>ATP is formed by oxidative phosphorylation</td>
</tr>
<tr>
<td>7. H-carrier involved</td>
<td>NADP</td>
<td>NAD and FAD</td>
</tr>
<tr>
<td>8. Metabolic process</td>
<td>a) anabolism b) overall is a reduction process</td>
<td>a) catabolism b) overall is a oxidation process</td>
</tr>
</tbody>
</table>

Compensation point:-
- It is the point of the light intensity at which the rate of photosynthesis is exactly balanced by the combined rates of respiration, so that net exchange of O₂ and CO₂ is zero.
- At normal daylight intensities, the rate of photosynthesis exceeds respiration, so the compensation points occur twice a day = one at morning, the other before the sunset.
- Usually shade plants tend to reach their light compensation points faster than sun plants since their chlorophyll content are rich and able to undergo rapid photosynthesis at dim light.

Fig.8 Graph showing the effect of light intensity on the rate of photosynthesis, as measured by the amount of CO₂ exchange.
Compensation period: -
- it is the time taken for a plant to reach its compensation point from a complete darkness condition to a fully illuminated place
- the length of compensation period varies with different plants, shade plants generally have a shorter compensation period since their ability to utilize dim light is great

Exercise: (92 I 11)
‘Maximum photosynthesis’ refers to the highest possible rate of photosynthesis attainable by a plant species under optimal conditions. The graph below shows how the light intensity affects the rates of photosynthesis of different plant types (measured as percentage of their maximum photosynthesis): 

(a) Different plant types may have different ABSOLUTE values of ‘Maximum photosynthesis’. Suggest one reason for such differences. [2 marks]
(b) Describe and explain the effect of light intensity on photosynthesis of the tree species A. [4 marks]
(c) With reference to the natural habitat of the green algae, explain how they differ from tree species A in the following:
(i) the optimal light intensity for photosynthesis
(ii) the effect of a light intensity of 1 kJ m\(^{-2}\) min\(^{-1}\) on photosynthesis [4 marks]
(d) It is known that tree species B will not grow well when planted together with tree species A. Suggest a possible explanation with reference to the graph above. [3 marks]
(e) Diatoms, a type of phytoplankton, are known to exhibit daily vertical migration (moving down at noon). Given that normal light intensity at noon at the sea surface ranges from 1.0 to 2.5 kJ m\(^{-2}\) min\(^{-1}\), suggest a likely advance of this phenomenon. [3 marks]
(f) Explain how two other environmental factors may affect the rate of photosynthesis of plants [4 marks]
F. Measuring the rate of photosynthesis:

a) Changes in dry weight
- if portions of a leaf are removed with a cork borer and heated to constant weight and then similar portions taken after the plant has been photosynthesizing, the “apparent gain” in weight is partly due to photosynthesis
- respiratory losses over the same period have to be measured and added to the apparent gain then the photosynthetic rate of the plant has been estimated

b) Measure the CO₂ uptake
1. Titration method
   - CO₂ uptake is measured by passing a stream of air over a plant in a closed container and then bubbling a sample of the “used” air through an alkaline solution
   - titration of the alkaline solution would reveal how much CO₂ was dissolved in the solution
   - this could be compared with a control and the amount of CO₂ taken up by the plant was calculated
2. Infra-red absorption method
   - a new method is known as infra-red absorption by CO₂
   - this method make use of the ability of CO₂ absorb certain wavelength of infra-red
   - the intensity of the absorption bands decrease as CO₂ concentration decreases
   - this method has the advantage of giving instantaneous recording of the CO₂ concentration in a stream of air passing over a plant in a closed vessel

c) Measuring the O₂ production
- the following two methods, corrections must be made for gaseous exchange due to respiration
1. Bubble counting
   - this method is suitable to measure the photosynthetic rate of the aquatic plants e.g. Hydrilla
   - bubbles of O₂ are given from the cut end of the stem of Hydrilla when it is immersed in illuminated pond water or a diluted NaHCO₃ solution
   - the number of bubbles of O₂ given off in a time is an indication of the rate of “apparent photosynthesis” of the plant
   - however, there are drawbacks
     - irregularity of the size of bubbles
     - hard to measure a chain of bubbles
     - time consumable

![Fig.9 Investigating the evolution of oxygen during photosynthesis](image-url)
2. Volume measuring
   - $O_2$ is collected in a graduated capillary tube
   - this can be used in comparative means of the plants at various conditions
   - it can also measure the effect of temperature, light intensity and $CO_2$ concentration on the rate of photosynthesis

   ![Apparatus for measuring the rate of oxygen evolution by a water plant during photosynthesis](image)

   **Fig.10** Apparatus for measuring the rate of oxygen evolution by a water plant during photosynthesis.

   d) Use of radioactive isotope (C$^{14}O_2$):
   - decrease in the radioactivity of the introduced sample of C$^{14}O_2$ over a period of time would give a very accurate description of the photosynthetic rate
   - the amount of C$^{14}O_2$ taken up can be measured directly for radioactivity by an analysis of the plant material involved

**Exercise : (93 II 3a)**
Give the chemical equation for photosynthesis. From the equation, what changes in the substances taken up and produced might be used to measure the rate of photosynthesis ?. [3 marks]
Syllabus: The general nature of chemosynthesis using nitrifying bacteria as an example.

Chemosynthesis (chemoautotrophic nutrition)

Chemosynthetic organisms are bacteria using CO₂ as a carbon source and obtaining their energy from chemical reactions rather than light. The energy is obtained by oxidizing inorganic materials such as H₂, H₂S, S, Fe²⁺, NH₃ and NO₂. Chemosynthetic bacteria play important roles in the biosphere, principally in maintaining soil fertility through their activities in the nitrogen cycle.

I. Nitrifying bacteria :-
- certain nitrifying bacteria are able to oxidize the ammonia released by saprophytic organisms into nitrites, usually in the form of nitrous acid (HNO₂)
- other members of this group can convert the nitrites to nitrates (NO₃⁻)
- the bacteria work aerobically and increase the soil fertility
  i) Nitrosomonas: it converts ammonia to nitrite aerobically
     \[ 2 \text{NH}_3 + 3 \text{O}_2 \rightarrow 2 \text{HNO}_2 + 2 \text{H}_2\text{O} + \text{energy} \]
  ii) Nitrobacter: it changes nitrites to nitrates aerobically
     \[ 2 \text{HNO}_2 + \text{O}_2 \rightarrow 2 \text{HNO}_3 + \text{energy} \]

II. Iron bacteria :-
- they make use of various iron compounds as source of energy
- they are the organisms responsible for the brownish deposits sometimes formed at the bottom water containers
  \[ 4 \text{FeCO}_3 + \text{O}_2 + 6 \text{H}_2\text{O} \rightarrow 4 \text{Fe(OH)}_3 + 4 \text{CO}_2 + \text{energy} \]

III. Colourless sulphur bacteria :-
- it is able to oxidize sulphur in the presence of oxygen to release energy
  \[ 2 \text{S} + 3 \text{O}_2 + 2 \text{H}_2\text{O} \rightarrow 2 \text{H}_2\text{SO}_4 + \text{energy} \]
- in some anaerobic species, they can use nitrite as a hydrogen acceptor, thus carrying out denitrification
  \[ 5 \text{S} + 6 \text{HNO}_3 + 2 \text{H}_2\text{O} \rightarrow 5 \text{H}_2\text{SO}_4 + 3 \text{N}_2 + \text{energy} \]