

1. Details of Module and its structure

Module Detail	
Subject Name	Biology
Course Name	Biology 02 (Class XI, Semester - 2)
Module Name/Title	Early Experiments: Part – 1
Module Id	kebo_21301
Pre-requisites	Basic information of Photosynthesis
Objectives	After going through this lesson, the learners will be able to understand the following: <ul style="list-style-type: none">• Photosynthesis and the experiments done by:• Joseph Priestley and bell-jar experiments• Jan Ingenhousz• Jean Senebier• Nicolas Theodore de Saussure• Julius von Sachs• Theodor Wilhelm Engelmann• Frederick Frost Blackman• Cornelius Bernardus van Niel
Keywords	Photosynthesis, Experiments in photosynthesis

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1. Photosynthesis

Photosynthesis is synthesis of organic compounds (food) with the help of light energy. It is a physico-chemical process carried out by green plants in the presence of sun light. In this process plants (green) convert carbon dioxide and water to carbohydrates (food) using light energy, and release oxygen. Photosynthesis is the greatest chemical factory on this earth producing millions of tons of solid plant material every day. Directly or indirectly, most organisms on this earth depend on photosynthesis for food and oxygen.



So, what is the mechanism of the process of photosynthesis? _

Module 1- Early Experiments

Introduction

The discovery of the process of photosynthesis has an interesting and a long history spanning over two centuries. Simple experiments by investigators trying to understand the growth and functioning of plants led to the unravelling of the mechanism of photosynthesis. Foremost among them are experiments carried out by Joseph Priestley, Jan Ingenhousz, Jean Senebier, Julius von Sachs, T.W. Engelmann, F. Blackman, Cornelius van Niel, R. Hill, Robert Emerson, Melvin Calvin, Daniel I. Arnon.

2. Joseph Priestley and bell-jar experiments

Between 1770 and 1772, Joseph Priestley, a British chemist and minister, carried out a series of experiments to study the effects of gases on plants and animals. He placed a burning candle inside an air-tight bell jar (Fig. 1a) and observed that the flame was extinguished within a few minutes (Fig. 1b) and it could not be lighted again without disturbing the setup. Similarly, when he placed a mouse inside the jar, the mouse died after a few minutes. He also found that a mouse suffocated and died (Fig. 1d) if placed inside a jar in which a candle had burned out (Fig. 1c) or a mouse had breathed and died earlier. Priestley concluded that burning of candle and breathing by mice or animals ‘injured’ the air or made it impure.



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In his next experiment, Priestley placed a sprig of mint plant inside the bell jar along with the burning candle (e). Though the candle burned for a longer time than in the previous experiment, the flame was extinguished (f). However, he was able to relight the candle which was inside the sealed jar with the mint twig again after seven days (g). Priestley came to the conclusion that the plant was able to ‘refresh’ or purify the air ‘injured’ by the burning candle and breathing of animals.

Priestley made an important observation that, “The injury which is continually done by such a large number of animals is, in part at least, repaired by the vegetable creation.”

In 1774, Priestley chemically produced a gas (oxygen) which made candles burn brighter and mice more active but he did not relate it with the plant’s ability to ‘purify’ air. Antoine Lavoisier named the gas “Oxygen” in 1777. Lavoisier also showed that the ‘fixed air’ or ‘impure air’ is composed of carbon and oxygen, and is carbon dioxide (CO₂).

Plants → **Purify air**

3. Jan Ingenhousz

Jan Ingenhousz, a Dutch biologist, was greatly influenced by Priestley’s findings and started repeating his experiments. In 1779, while working with aquatic plants, he observed that gas bubbles were given off from green parts of the plant in the presence of light but no bubbles formed when the plant was placed in shade or in darkness. He identified the gas in the bubbles as oxygen. He, thus, concluded that in the dark, plants behave like animals and make the air impure.



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Therefore, it was Ingenhousz who established the key facts that green parts of plants produce oxy-
gen in the presence of light.

Green plant parts $\xrightarrow{\text{light}}$ **Oxygen**

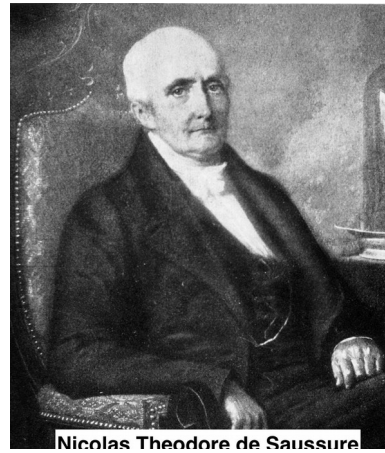
4. Jean Senebier

A Swiss botanist, Jean Senebier was intrigued by another finding of Jan Ingenhousz (1780), that is, plants present in boiled distilled water which lacked the impure air did not form bubbled even in the presence of sunlight. He repeated the experiment and observed that more bubbles or oxygen got liberated by plants in water saturated with 'impure' air. Senebier (1782) reasoned that plants need impure air to produce oxygen.

In 1783, Pierre Laplace demonstrated that carbon stored in the body burns with oxygen in air to produce carbon dioxide in exhaled air.

Subsequently, Senebier in 1788, concluded that carbon dioxide is consumed by plants in the production of oxygen, and that carbon in carbon dioxide is used by plants as nutriment.

Carbon dioxide $\xrightarrow{\text{light}}$ **Plant mass + Oxygen**
green plant parts

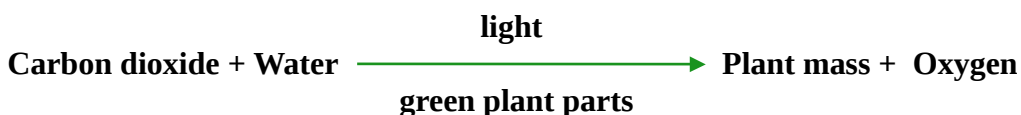


Source: https://upload.wikimedia.org/wikipedia/commons/thumb/0/0b/Jean_Senebier.jpg/1200px-Jean_Senebier.jpg

Source: https://upload.wikimedia.org/wikipedia/commons/a/a0/Nicolas-Théodore_de_Saussure.jpg

5. Nicolas Theodore de Saussure

N.T. de Saussure, a Swiss plant physiologist, carried out controlled experiments using scientific methods. He enclosed plants in air tight glass containers and not only weighted the plant but also quantified the gases present inside before and after the experiments. He proved that the amount of carbon dioxide consumed by plants is approximately equal to the amount of oxygen liberated. De Saussure also noticed that the weight of carbon absorbed was less than the increase in weight of the plant. In 1804, he deduced that since water is also absorbed by plants, both carbon dioxide and water must contribute to the formation of the plant body mass.



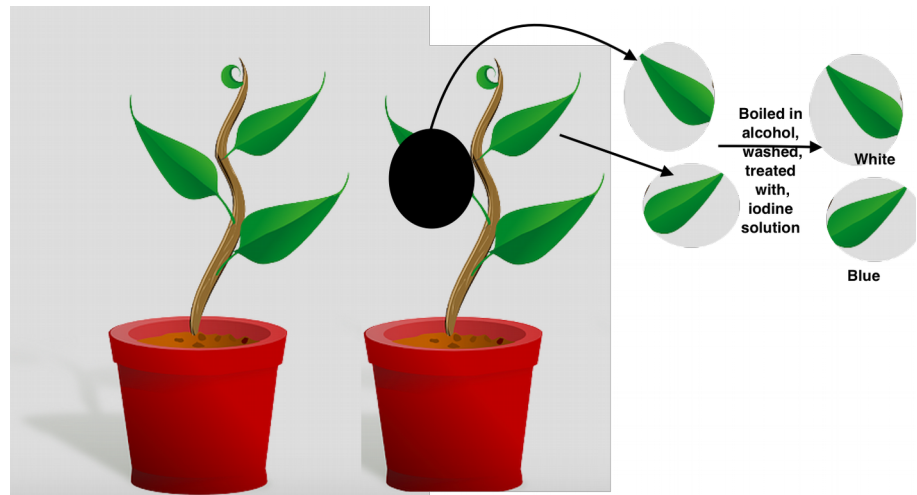
In 1817, Joseph Bienaimé Caventou and Pierre Joseph Pelletier isolated and named the green pigment from plant as “**chlorophyll**”. However, it was Hugo von Mohl in 1837, who described the discreet bodies within green plant cells as ‘**chlorophyll grains**’ which contain chlorophyll. Later, in 1883, the ‘chlorophyll grain’ was named as “**chloroplastid**” by A. F. W. Schimper and adopted as “**chloroplast**” by E. Strasburger (1884).

6. Julius von Sachs

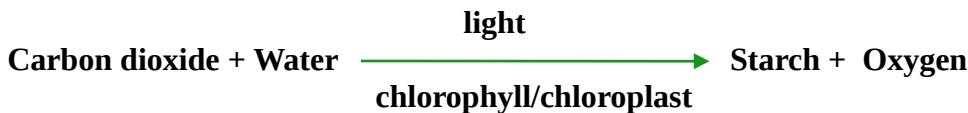
In the 1860s, Julius von Sachs, a German botanist, carried out experiments (routinely done in classrooms now to demonstrate photosynthesis) which helped to present the overall reaction of photosynthesis. He found that a leaf exposed to sunlight, then bleached white and treated with iodine turned blue-black indicating the presence of starch, whereas a leaf of the same plant not exposed to light remained white indicating the absence of starch. He concluded that starch is the

first product of photosynthesis and is formed by absorption of carbon dioxide in the presence of light. He also confirmed the presence of chlorophyll inside special bodies, the 'chlorophyll grains' and demonstrated that the starch grains formed from inorganic matter only inside 'chlorophyll grains'.

Sachs is, therefore, credited with finding the involvement of chlorophyll in fixing carbon dioxide into starch in 'chlorophyll grains' in the presence of light during photosynthesis.



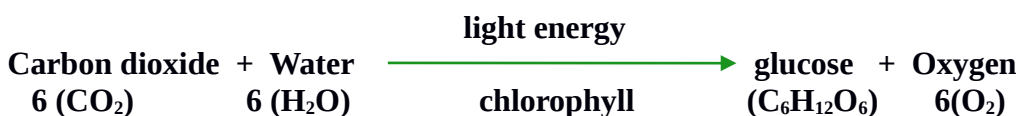
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He noted that starch is necessary for plant growth, and that starch which accumulates in leaves during the day is dissolved and converted to other forms such as sucrose at night for transport to other parts of plant where it is used for growth and development.

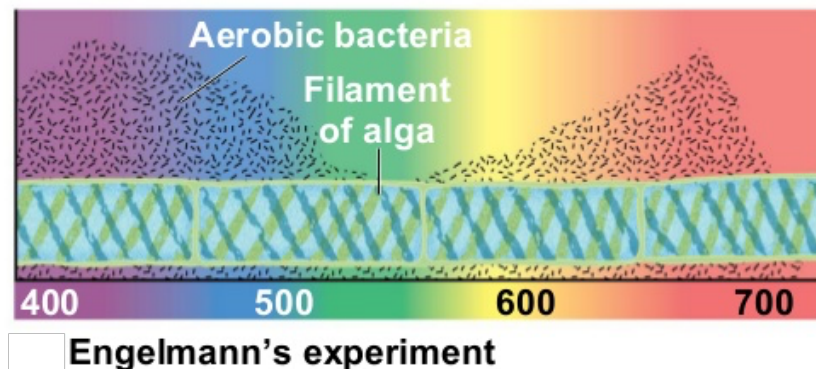
Since starch is made of glucose, the above equation was rewritten as



7. Theodor Wilhelm Engelmann

The next important contribution to understanding of photosynthesis was made by a German botanist, T. W. Engelmann. In 1881, he found that when he placed a strand of Spirogyra along with bacteria in a drop of water and flashed light, the bacteria moved and accumulated near the spiral

chloroplasts. He concluded that the aerobic bacteria moved toward chloroplasts as they were releasing oxygen. The next year (1882) he performed a similar experiment to address the question whether green plants produce oxygen equally well under all the wavelengths of light. He used a micro-spectroscope, a microscope with a prism that could split light and focus the spectrum of light on the stage of the microscope. He was able to expose different regions of a strand of Cladophora to different wavelengths of light in the micro-spectroscope. He observed the presence of highest concentration of bacteria near the regions of red and blue-violet light, and concluded that these wavelengths of light produced more oxygen than the other wave lengths and therefore, must be photosynthetically the most efficient.



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(M e a s u r e d b y a m o u n t o f o x y g e n r e l e a s e d)



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This is the first demonstration of action spectrum of photosynthesis. An action spectrum is the rate of a process or activity at different wavelengths of light and is generally represented graphically. The wave length of light which most effectively drives the process can be deduced from such a graph.

Action spectrum of Photosynthesis.

Rate of photosynthesis is maximum at the violet-blue and the red wave lengths and minimum in the green wave lengths.

The term Photosynthesis, first proposed by Charles Barnes, came into being only in 1893.

8. Frederick Frost Blackman

A British plant physiologist, Frederick Blackman studied the various factors affecting photosynthesis and demonstrated in 1905, that photosynthesis which is regulated by a number of factors such as light, temperature and carbon dioxide concentration followed the “Law of limiting factors”. The rate of photosynthesis is limited by the factor which is the slowest or least.

Another major conclusion drawn from Blackman’s experiments is that photosynthesis is a two step process; a light energy capturing step (**light reactions or light-dependent reactions**) and a step which does not directly require light but involves biochemical reactions (**dark or light-independent reactions**).

9. Cornelius Bernardus van Niel

While studying anaerobic purple sulfur and green sulfur bacteria, a Dutch-American microbiologist, Cornelius van Niel, discovered in 1931, that these photosynthetic bacteria used hydrogen sulphide (H₂S) in place of water (H₂O) and released sulphur or sulphur compounds instead of oxygen. He concluded that these bacteria and green plants split H₂S and water respectively, with the help of light energy and release energy and hydrogen which would then be used to reduce carbon dioxide to carbohydrates.



where **CH₂O** represents a carbohydrate and **A** is the product of oxidation which is oxygen in green plants and sulfur in the above photosynthetic bacteria. This meant that all the oxygen released during photosynthesis is the result of splitting of water molecules by light energy, and does not evolve from carbon dioxide as thought earlier. Later, Samuel Ruben, Martin Kamen and colleagues in 1941, confirmed van Niel’s theory using a heavy isotope of oxygen, ¹⁸O. ¹⁸O was used to label both water (H₂¹⁸O) and carbon dioxide (C¹⁸O₂), which were provided separately to plants. They found that when plants were watered with H₂¹⁸O, they produced ¹⁸O₂ but when C¹⁸O₂ was provided, plants produced normal O₂ indicating that oxygen released during photosynthesis comes from water and not from carbon dioxide.

The reaction of photosynthesis in green plants was then modified and expressed as



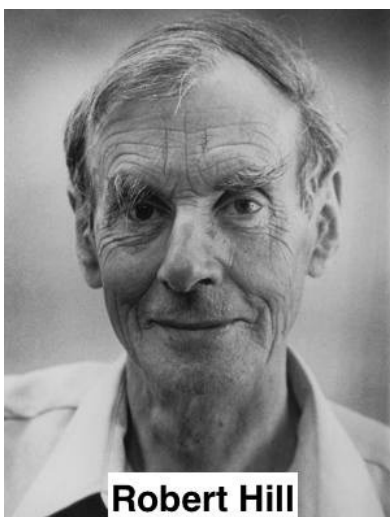
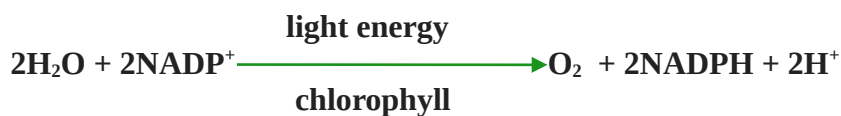
10. Robert Hill

Robert Hill, also known as Robin Hill, a British biochemist, discovered in 1937, that isolated chloroplasts can evolve oxygen in the presence of sunlight and suitable electron acceptors such as ferricyanides. During the process no carbon dioxide was reduced.

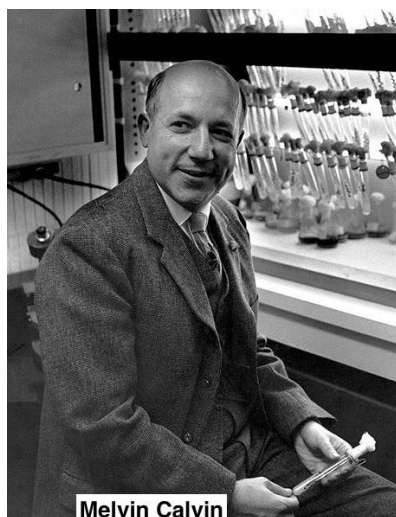
Thus, Hill's experiments provided evidence for the following:

- Photosynthesis is a two step process with separate light-dependent and light-independent (dark) reactions, supporting the conclusion from Blackman's experiments.
- Light reaction can occur independent of dark reaction.
- In the light reaction, also known as "**Hill Reaction**", light energy is used to split water evolving oxygen, hydrogen ions and electrons. The electrons are used to reduce a natural electron acceptor in the cells which was later discovered to be nicotinamide adenine dinucleotide phosphate (NADP⁺). Therefore, there is a transfer of electrons from water to an electron acceptor against a chemical potential gradient driven by light energy.
- Oxygen is produced in a process that is separate from the process that reduces carbon dioxide which is a light independent or "dark" process involving biochemical reactions.
- Oxygen evolves from water, confirming van Niel's finding.
- Whole cells are not required for some photosynthesis reactions; isolated chloroplasts can carry out partial reaction of photosynthesis.

Light Reaction



Robert Hill

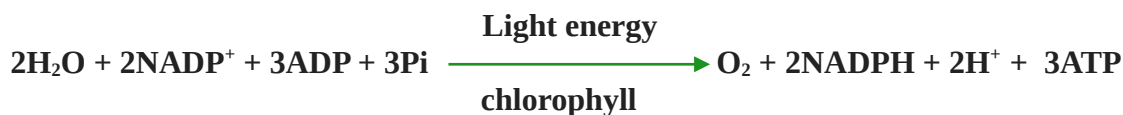


Melvin Calvin

Source: https://upload.wikimedia.org/wikipedia/en/2/2f/Robert_Hill_%28biochemist%29.jpg

Source: https://upload.wikimedia.org/wikipedia/commons/thumb/0/04/Melvin_Calvin.jpg/462px-Melvin_Calvin.jpg

Daniel I. Arnon (1954) discovered cyclic and non-cyclic photophosphorylation or ATP formation during photosynthesis.



Robert Emerson along with coworkers inferred from their red drop and enhancement effect experiments that two photosystems, PS I with a reaction centre chlorophyll a molecule - P₇₀₀, and PS II with a reaction centre chlorophyll a molecule - P₆₈₀, operate during light reaction. In 1960, R. Hill and F. Bendall suggested a scheme to link the two photosystems through cytochromes found in chloroplasts which has since been adopted to explain light reaction. Since the graphical representation of the scheme resembles the letter “Z”, it has been called the Z-scheme.

Light-independent (Dark) Reaction

Using radioactive isotope of carbon dioxide (¹⁴C), Melvin Calvin, James Bassham and Andrew Benson in 1953 discovered the light independent or carbon dioxide reduction reactions (also called the Calvin cycle) in which the high-energy products formed in the light reactions are used to reduce carbon dioxide to carbohydrates.



11. Summary

It took almost a period of two centuries to elucidated the process of photosynthesis. Initial experiments by Joseph Priestley (1771) indicated that plants purify air made impure by animals. The role of sunlight and green parts of the plants in purifying air by releasing oxygen was demonstrated by Jan Ingenhousz. Later Jean Senebier concluded that carbon dioxide is needed by plants for the production of oxygen, and that carbon in carbon dioxide is used by plants to make food. The requirement of water by green plants, in addition to carbon dioxide, to synthesize food in the presence of sunlight was deduced by De Saussure through quantitative analysis. Julius von Sachs found starch to be the first visible product of photosynthesis and emphasised the role of light, carbon dioxide and chlorophyll in the synthesis of starch. The action spectrum of photosynthesis was first performed by T. W. Engelmann (1882) who found that photosynthesis is most efficient in red and blue-violet light. Photosynthesis as a two-step process with light dependent and light independent (dark) steps or reactions was revealed by F. F. Blackman’s experiments in 1905.

Investigations by C. B. van Niel, Robert Hill and D. I. Arnon proved that in light reactions, light energy is used to split water in the presence of chlorophyll, to release oxygen and form high energy molecules, NADPH and ATP. The participation of two photosystems in light reactions was established by R. Emerson.

Melvin Calvin, James Bassham and Andrew Benson are credited with the discovery of the dark or light-independent reactions, in 1953, where carbon dioxide is reduced to carbohydrates.