

 **SL IB Biology**

## Photosynthesis

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- \* Absorption & Action Spectra: Skills
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## The Process of Photosynthesis



Your notes

### Transformation of Light Energy During Photosynthesis

- **Simple, inorganic compounds** are converted into complex organic ones by photosynthesis
  - The energy required is provided by **light**
- Photosynthesis occurs in autotrophic organisms such as **plants, algae** and **cyanobacteria**
- Photosynthesis is a form of **energy conversion**, from **light energy** to **chemical energy**, stored in biomass
- **Energy is stored** within the bonds of these organic compounds and provides most of the **chemical energy** needed for **life processes** in ecosystems

#### Examiner Tip

Remember, energy is never created or destroyed; it is only ever converted from one form to another!

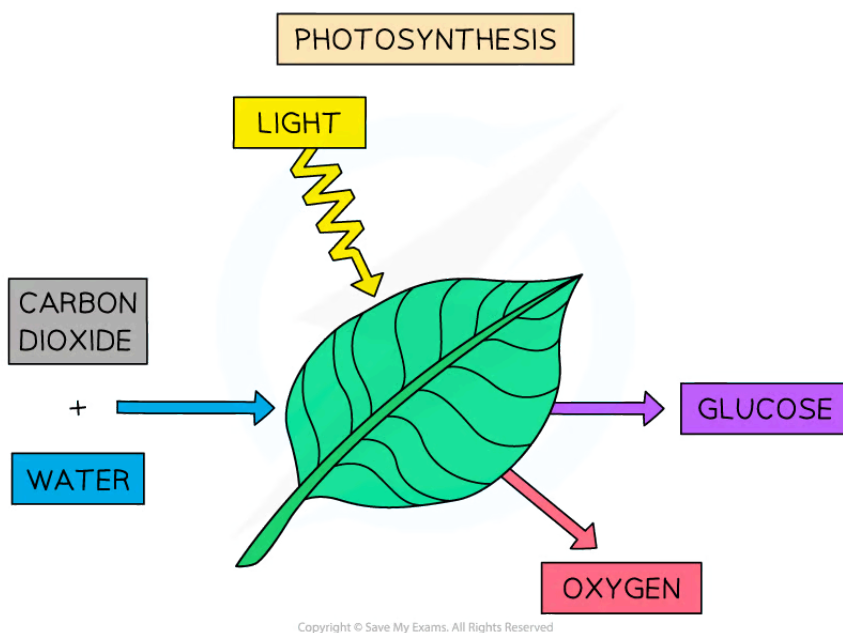


Your notes

## Conversion of Carbon Dioxide to Glucose

- During photosynthesis, **carbon dioxide** is converted to **glucose** using hydrogen released when a water molecule is split
  - Oxygen is released as a waste product
- The **reactants** of photosynthesis are **carbon dioxide** and **water**
- The **products** of photosynthesis are **glucose** and **oxygen**

### Reactants and products of photosynthesis diagram



### Photosynthesis as it takes place in a leaf

- We can represent this chemical reaction in a word equation

### Photosynthesis word equation



### A word equation to represent photosynthesis



### Examiner Tip

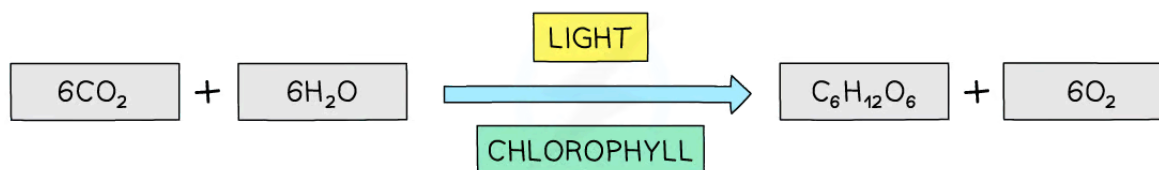
The glucose and oxygen formed during photosynthesis are the reactants of aerobic cell respiration while carbon dioxide and water released during respiration are used as the reactants of photosynthesis

- Respiration is the process by which **energy is released** from organic molecules in living cells

## Release of Oxygen

- Photosynthesis is carried out in **plants, algae and cyanobacteria**
- The **oxygen** that is released comes from the **water splitting** process which also provides **hydrogen** to allow the synthesis of **glucose**
- The paths of the oxygen and hydrogen can be seen more clearly when looking at the **chemical symbol equation** for photosynthesis

### Chemical symbol equation for photosynthesis



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### Examiner Tip

Note that you are only expected to know the word equation for photosynthesis for exam purposes



Your notes

## Separating Photosynthetic Pigments: Skills

### Separating Photosynthetic Pigments: Skills

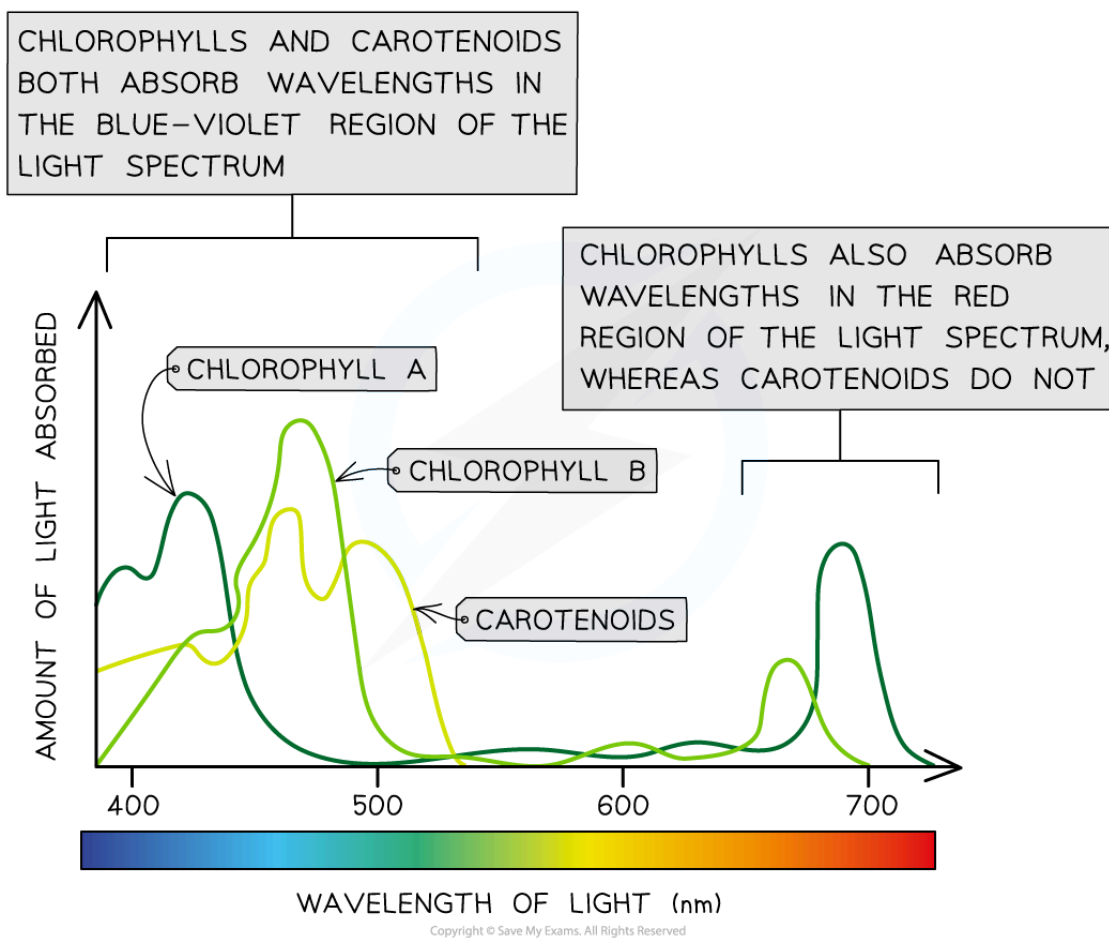
#### Separation of photosynthetic pigments by chromatography

- Plants contain several different **photosynthetic pigments**, which **absorb different wavelengths of light**
- There are **two groups** of pigments: **chlorophylls** and **carotenoids**
- Carotenoids surround the chlorophyll and absorb both similar and different wavelengths of light to chlorophyll
  - This **expands the range of wavelengths** that can be absorbed from light for use in photosynthesis

**Chloroplast Pigments Table**

| Pigment group | Name of pigment                 | Colour of pigment             |
|---------------|---------------------------------|-------------------------------|
| Chlorophylls  | Chlorophyll a<br>Chlorophyll b  | Blue- green<br>Yellow - green |
| Carotenoids   | $\beta$ carotene<br>Xanthophyll | Orange<br>Yellow              |

- Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
  - They reflect green light, causing plants to appear green
- Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum



**Chlorophyll and carotenoids absorb light across the visible light spectrum to use in the light-dependent reaction of photosynthesis**

## Chromatography

- **Chromatography** is an experimental technique that is used to **separate mixtures**
  - **Different components** within the mixture travel through the material at **different speeds**
  - This causes the different components to **separate**
  - A retardation factor ( **$R_f$  value**) can be calculated for each component of the mixture
- Two of the most common techniques for separating these photosynthetic pigments are:
  - **Paper chromatography** - the mixture of pigments is passed through paper (cellulose)
  - **Thin-layer chromatography (TLC)**- the mixture of pigments is passed through a thin layer of adsorbent (eg. silica gel), through which the mixture travels faster and separates more distinctly
- Paper chromatography can be used to separate photosynthetic pigments although **TLC gives better results**

## Apparatus



Your notes

- Leaf sample
- Distilled water
- Pestle and mortar
- Filter paper
- Capillary tube
- Chromatography solvent
- Propanone
- Pencil
- Ruler

## Method

- Draw a straight line in pencil approximately 1cm above the bottom of the filter paper being used
  - **Do not use a pen** as the ink will separate into pigments within the experiment and obscure the results
- Cut a section of leaf and place it in a mortar
  - It is important to **choose a healthy leaf** that has been in direct sunlight so you can be sure it contains many active photosynthetic cells
- Add 20 drops of propanone and use the pestle to grind up the leaf sample and release the pigments
  - Propanone is an organic solvent and therefore fats, such as the lipid membrane, dissolve in it
  - The combination of propanone and mechanical pressure breaks down the cell and chloroplasts to **release the pigments**
- Extract some of the pigment using a capillary tube and spot it onto the centre of the pencil line you have drawn
- Suspend the paper in the chromatography solvent so that **the level of the solvent is below the pencil line** and leave the paper until the solvent has reached the top of the paper
  - The mixture is **dissolved** in the **solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
- Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to
  - The pigment should have separated out and there should be different spots on the paper at different heights above the pencil line, these are the separate pigments
- Calculate the  $R_f$  value for each spot

$$R_f \text{ value} = \frac{\text{distance travelled by component (pigment)}}{\text{distance travelled by the solvent}}$$

- Always measure to the centre of each spot

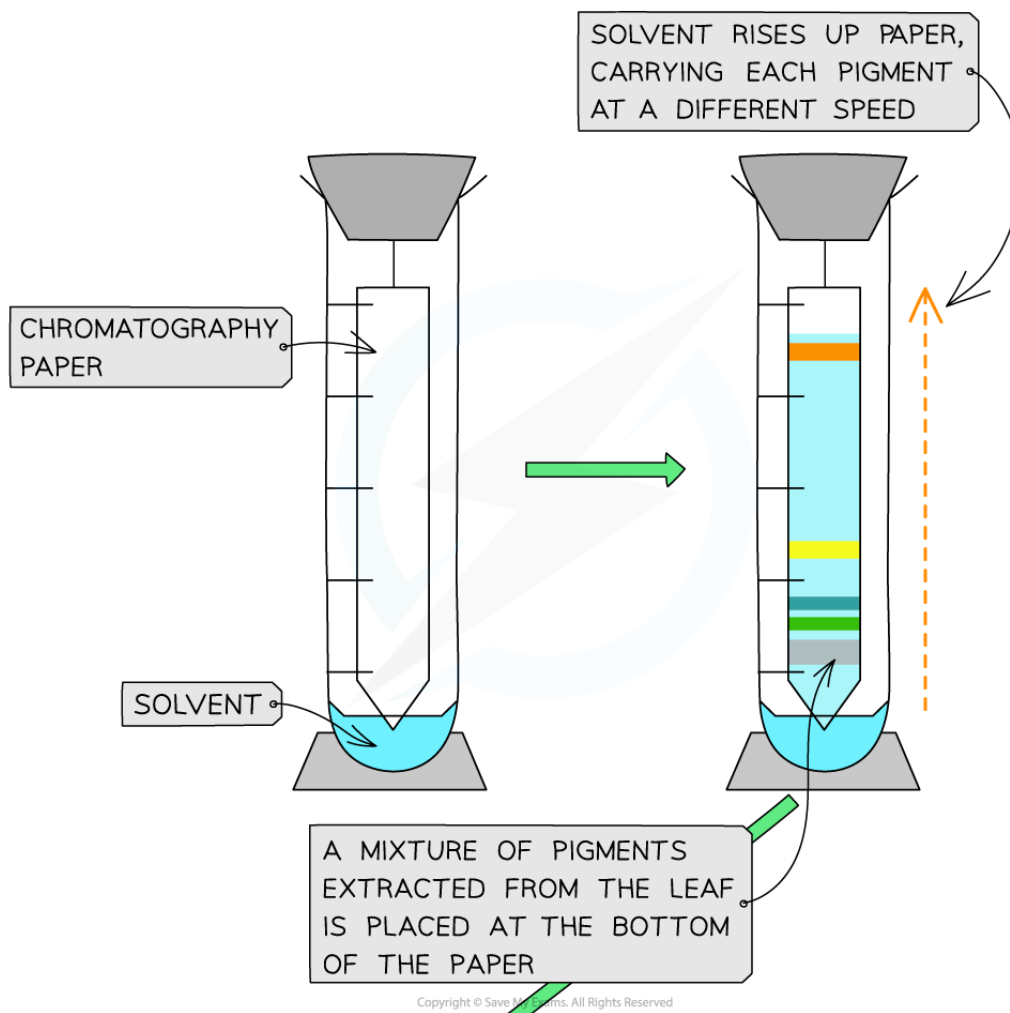
## Results

- Chromatography can be used to **separate and identify chloroplast pigments** that have been extracted from a leaf as each pigment will have a unique  $R_f$  value

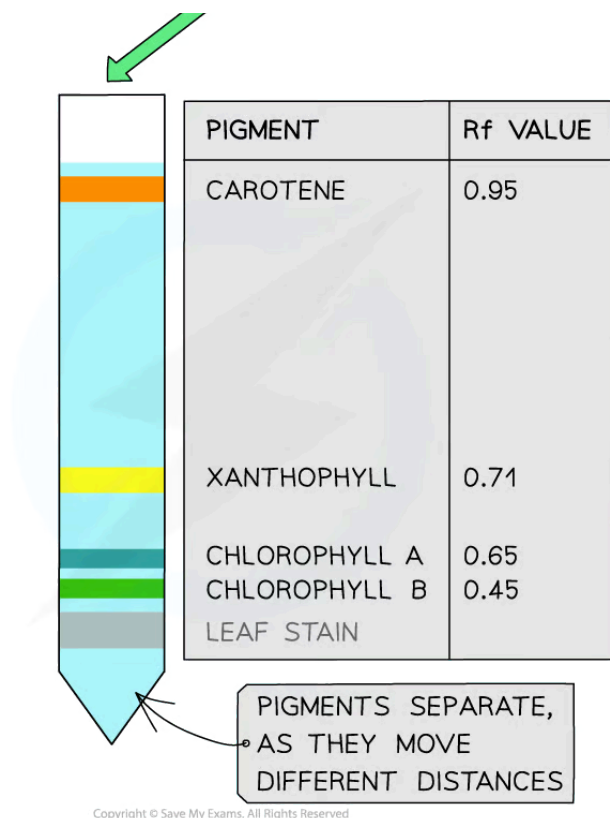


Your notes

- The  $R_f$  value demonstrates how far a dissolved pigment travels through the stationary phase
  - Molecules with a higher affinity to the stationary phase, such as large molecules, will travel slower and therefore have a **smaller  $R_f$  value**
  - Molecules that are more soluble in the mobile phase will travel faster and therefore have a **larger  $R_f$  value**
- Although specific  $R_f$  values depend on the solvent that is being used, in general:
  - **Carotenoids** have the **highest  $R_f$  values** (usually close to 1)
  - **Chlorophyll b** has a **much lower  $R_f$  value**
  - **Chlorophyll a** has an  $R_f$  value somewhere **between** those of carotenoids and chlorophyll b
  - **Small  $R_f$  values** indicate the pigment is **less soluble** and/or **larger** in size







**Paper chromatography is used to separate photosynthetic pigments. These pigments can be identified by their  $R_f$  values. In this example, a line of the mixture (rather than a spot) is added to the paper.**

### Limitations

- Paper chromatography is not as specific as other chromatography techniques
  - It is sufficient to separate and distinguish different pigments and to calculate their  $R_f$  value
- Chromatography does not give data on the amount of each pigment present or the wavelengths that they absorb
  - Colorimetry can be used to calculate these values

### Examiner Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*. You don't have to remember specific  $R_f$  values, just know that they differ between each type of pigment.

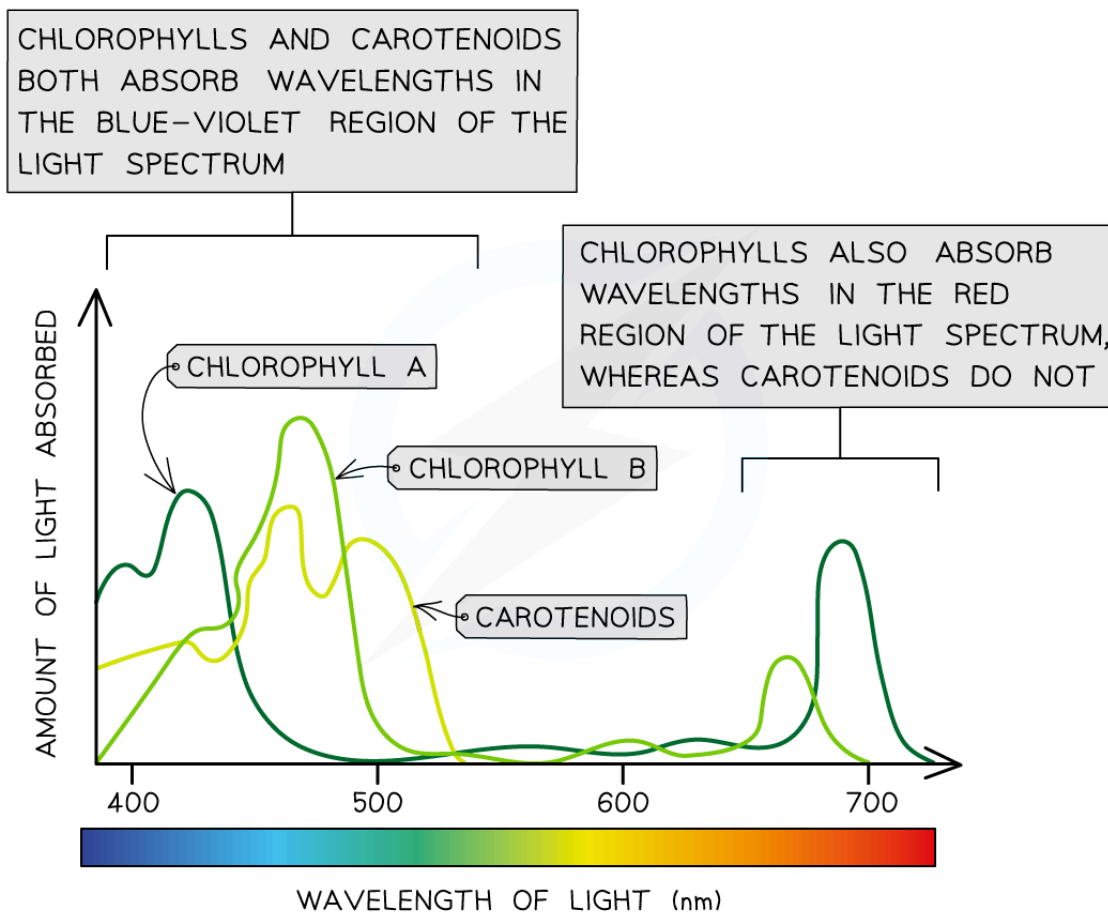


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## Absorption Spectra

### Absorption Spectra

- Light is made up of a mixture of all the **visible wavelengths** to include red, orange, yellow, green, blue, indigo and violet
- An **absorption spectrum** is a graph that shows the **absorbance** of different wavelengths of light by a particular pigment in the chlorophyll
- Within the chlorophyll, light energy results in the **excitation of electrons** which triggers transfer of electrons leading to a series of reactions which make up the process of **photosynthesis**
  - During photosynthesis, **light energy is transformed to chemical energy** when glucose is formed
- Chlorophylls** absorb wavelengths in the **blue-violet and red regions** of the light spectrum
- Carotenoids** absorb wavelengths of light mainly in the **blue-violet region** of the spectrum
- The chemical structure of these molecules determines the wavelengths of light that can be absorbed
- The green part of the spectrum is largely **reflected** from the leaf and this is why leaves usually appear green



*Absorption spectra of chlorophyll A, chlorophyll B and carotenoid pigments*



Your notes



Your notes

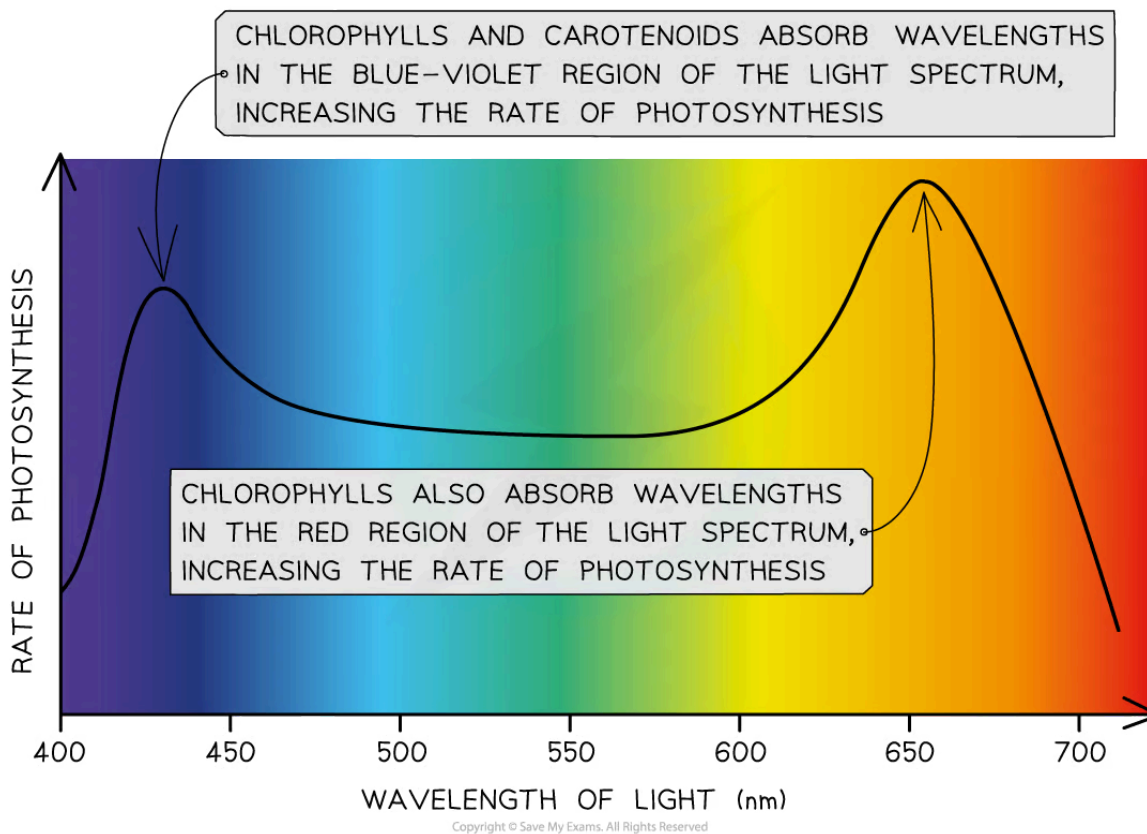
## Absorption & Action Spectra: Skills

### Comparing Absorption & Action Spectra

#### What is an action spectrum?

- An **action spectrum** is a graph that shows the **rate of photosynthesis** at different wavelengths of light
- The rate of photosynthesis is **highest** at the **blue-violet** and **red** regions of the light spectrum, as these are the wavelengths of light that plants can **absorb** (i.e. the wavelengths of light that chlorophylls and carotenoids can absorb)

#### Diagram to show the action spectrum of chlorophyll pigments



The photosynthetic action spectrum shows the rate of photosynthesis at different wavelengths of light

#### Comparing action and absorption spectra

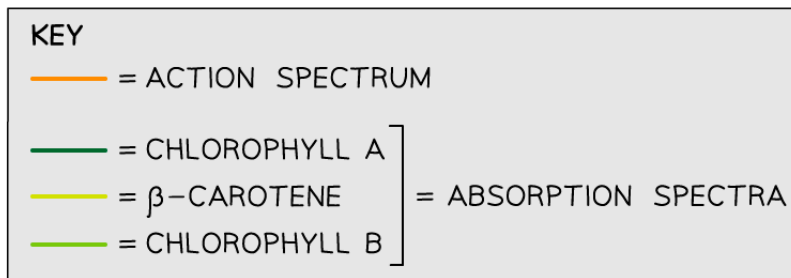
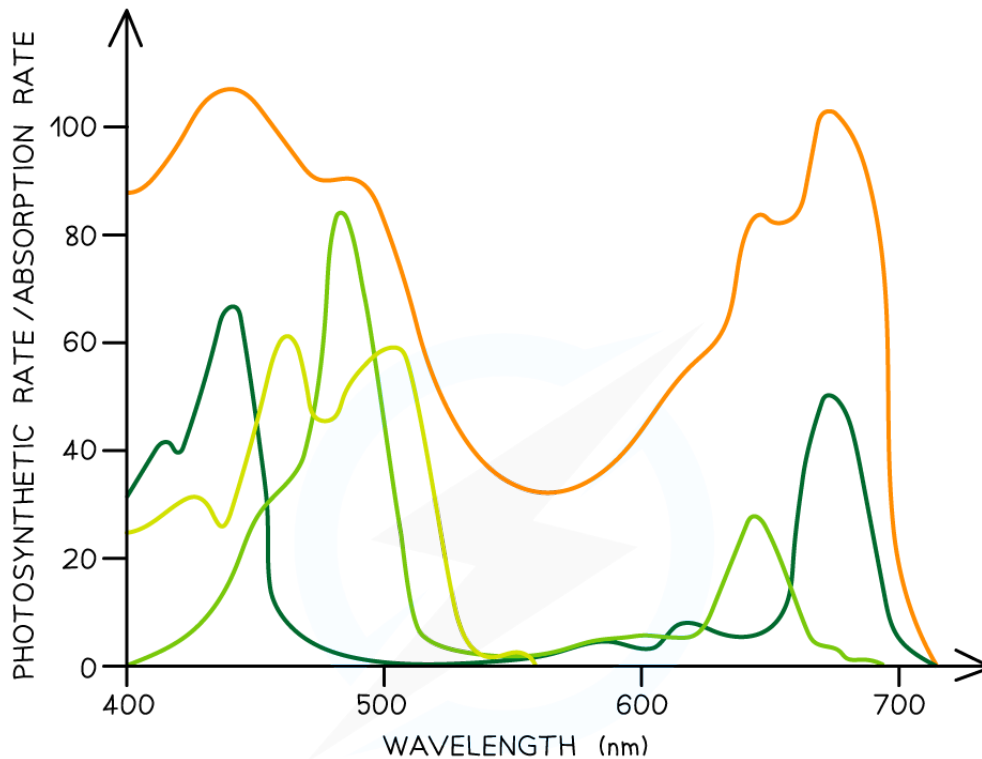
- There is a strong **correlation** between the cumulative absorption spectra of all pigments and the action spectrum:



Your notes

- Both graphs have **two main peaks** – at the **blue-violet** region and the **red** region of the light spectrum which supports the idea that the **most light energy** is absorbed at these wavelengths leading to the fastest rate of photosynthesis
- Both graphs have a **trough** in the **green-yellow** region of the light spectrum which supports the idea that the **least light energy** is absorbed at these wavelengths leading to the slowest rate of photosynthesis

**Diagram to show the correlation between action and absorption spectra**



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*An overlay of the photosynthetic absorption and action spectra shows there is a strong cumulative correlation*

### Determining the rate of photosynthesis



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- The rate of photosynthesis can be determined by measuring the **volume of oxygen produced** or the **carbon dioxide consumption** at different wavelengths of light
- An experiment can be set up similar to the one [investigating the effect of light intensity](#) on photosynthesis
  - Remember that the lamp should be **kept the same distance** from the pondweed as we are investigating the effect of different wavelengths of light only
  - Place different colour filters (covering the full light spectrum) in front of the lamp to change the colour of light the pondweed is exposed to
  - Measure the volume of oxygen produced or the number of bubbles released from the pondweed per minute for each colour
    - Include an experiment with no filter in front of the lamp to investigate the effect of white light on the rate of photosynthesis
  - Repeat the experiment several times to obtain reliable results

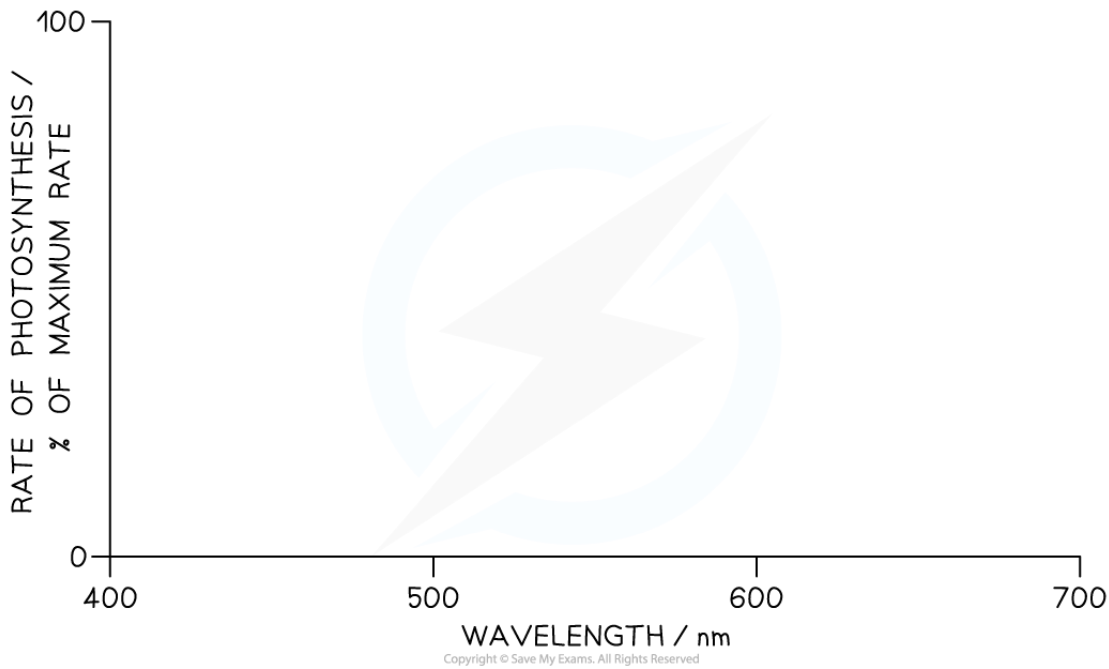
## Drawing an action spectrum for photosynthesis

### Step 1: Draw and label the axes

- Draw an x-axis
- Label the axis **wavelength**
- Add the units / **nm**
- Make 400 the smallest value and 700 the largest value
  - Label 500 and 600 nm on the x-axis
- Draw a y-axis
- Label it **Rate of photosynthesis / % of maximum rate**
- Make 0 the lowest value and 100 the highest value
  - No units are required because the y-axis is showing a percentage scale



Your notes



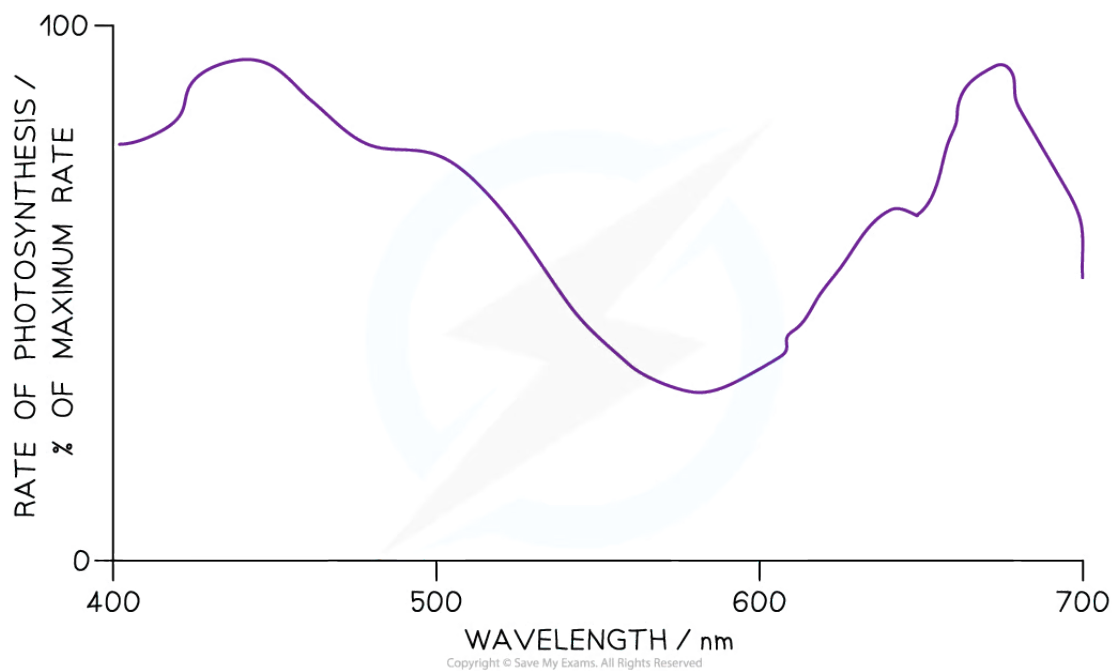
**Step 1: Draw and label the axes**

**Step 2: Draw the plot**

- There should be **two peaks of rate of photosynthesis**
  - One peak at either end, in the blue and red areas of the spectrum
  - And a **trough** in the middle, which represents green light
  - As below, with a smooth curve



Your notes



**Step 2: Sketch the Curve. An action spectrum for photosynthesis (colour range labels are not required)**

### Examiner Tip

Remember - the pigments themselves have a distinctive colour. This is different from the colours of light that they *absorb*. Key points to remember:

1. Label 400 - 700nm on the x-axis, in 100nm increments
2. Use a % scale on the y-axis
3. Smooth curve
4. Peaks at either end
5. Trough in the middle for green light





Your notes

## Limiting Factors of Photosynthesis: Skills

### Limiting Factors

- An **aquatic plant** such as *Elodea* or *Cabomba* is a good choice for investigating photosynthesis in plants, because the rate of photosynthesis can be measured by **counting oxygen bubbles** that come off a cutting of this plant
  - Oxygen output from terrestrial plants (that grow on land) would not be observable

### NOS: Hypotheses are provisional explanations that require repeated testing

- A hypothesis is a proposed explanation for an idea which may be true or false
- In an investigation the hypothesis can be tested through observations or experiments to provide either support or opposition to the proposed hypothesis
- The following investigation looks at the effect of limiting factors on the rate of photosynthesis
- A suggested **hypothesis** for an investigation into the effect of light intensity on photosynthesis could be:
  - **Light intensity will have an effect on the rate of photosynthesis**

### Identifying the variables in an investigation

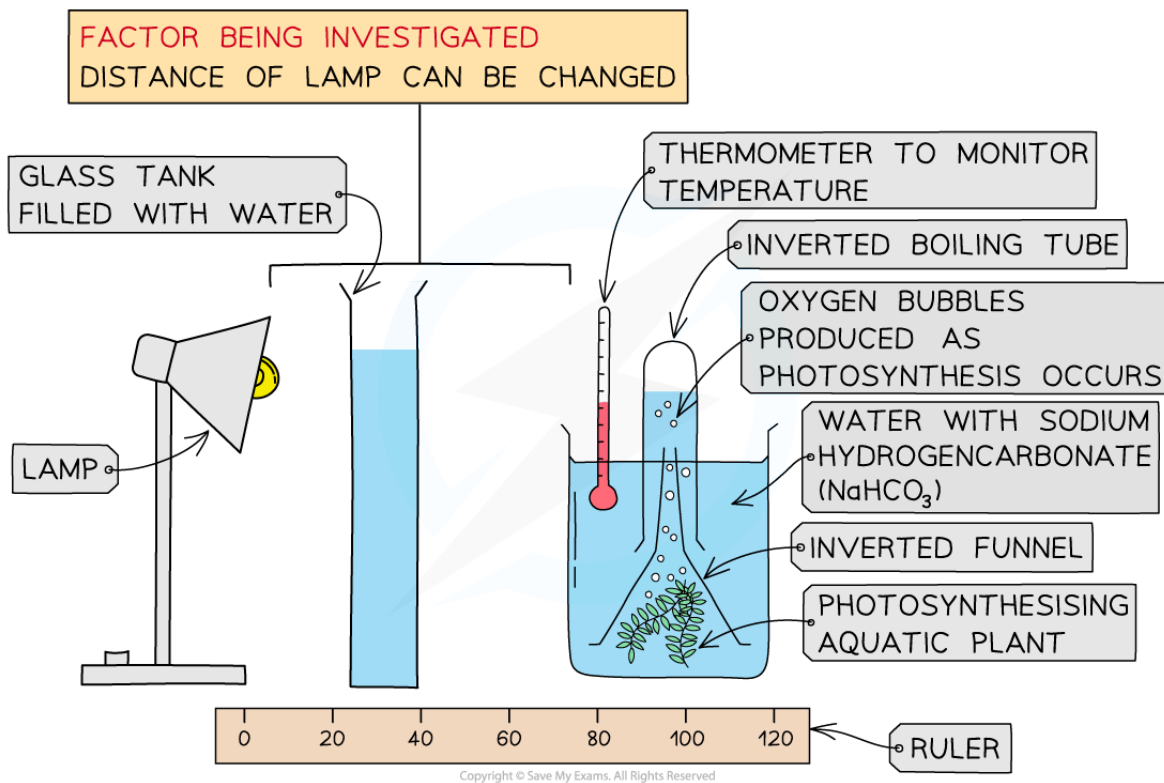
- When designing an experiment it is crucial that all variables (apart from the independent and dependent variables being investigated) are controlled
  - The independent variable is the factor that is **deliberately manipulated** between a specific range throughout the experiment
  - The dependent variable is the factor that is **measured** during the experiment (to see if it is affected by the changes to the independent variable)
  - Other variables must be **controlled** so that it can be said the independent variable is the only factor affecting the dependent variable during the experiment
- **Changes in light intensity, carbon dioxide concentration and temperature** are all limiting factors that affect the rate of photosynthesis and **can be altered experimentally** to measure the effect on the rate of photosynthesis
  - Any of these limiting factors could be selected as the **independent variable** in the investigation

### Effect of light intensity – experimental design

- Basic Experimental Setup
  - Aquatic plant cutting in water
  - Powdered sodium hydrogencarbonate ( $\text{NaHCO}_3$ )
  - Glass funnel
  - Boiling tube
  - Lamp for illumination
  - Glass tank filled with water



Your notes



### Measuring the effect of light intensity on the rate of photosynthesis in pondweed

#### Research Question

Does the rate of photosynthesis (number of bubbles released per min) of *Elodea* increase as the light intensity increases?

#### Method

- Place a piece of aquatic plant (*Elodea* or *Cabomba* are often used), into a beaker of water
- Place a lamp a set distance from the plant
- Record the number of bubbles observed in three minutes
- Repeat these steps for different distances between the lamp and plant

#### Improvements

- Use a **gas syringe** to collect and measure the volume of gas produced
- For **reliability** of data, **repeat** the experiment at least twice for each distance and calculate the mean number of bubbles
- Use of a **data logger** to measure results continuously

#### Variables to Be Controlled

- Temperature**
  - The glass tank filled with water absorbs any heat that is emitted from the lamp

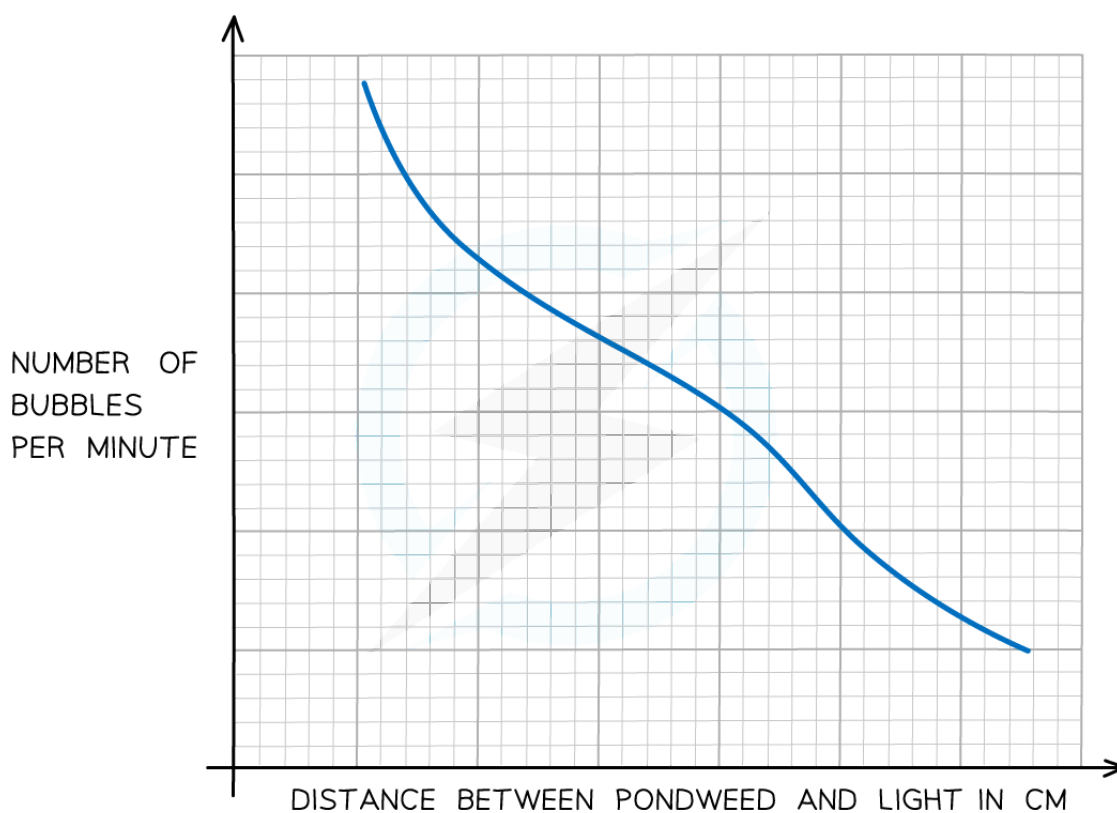


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- Modern LED bulbs can be used as they give off less heat than filament bulbs
- CO<sub>2</sub> concentration**
  - The water used around the plant is first **boiled and re-cooled** to remove any dissolved carbon dioxide
  - A **set mass of sodium hydrogencarbonate** is added to the water that surrounds the plant to make the concentration approx.  $0.1 \text{ mol dm}^{-3}$ 
    - This will ensure that the carbon dioxide concentration is not limiting the rate of photosynthesis

## Results

- A graph of the **number of bubbles produced per minute** against the **distance between the lamp and the plant** used can be drawn to see the pattern or trend
  - Distance between the lamp and the plant is linked to the light intensity



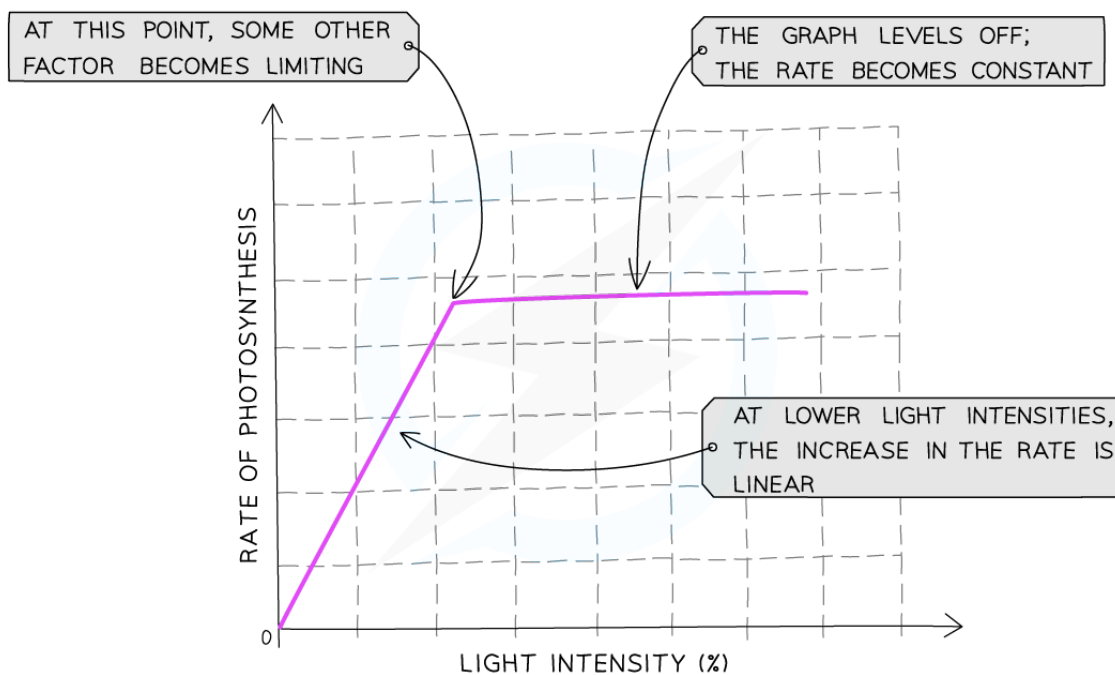
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### ***A graph of distance from the lamp against number of bubbles per minute***

- A graph can also be drawn showing the effect of different light intensities on the rate of photosynthesis
- It can be seen that:
  - As light intensity increases so too does the rate of photosynthesis (**positive correlation**)

- At this stage light intensity is the limiting factor
- At some point, there will be no further increase in the rate of photosynthesis if the light intensity is increased
  - Now temperature or carbon dioxide concentration may be limiting factors



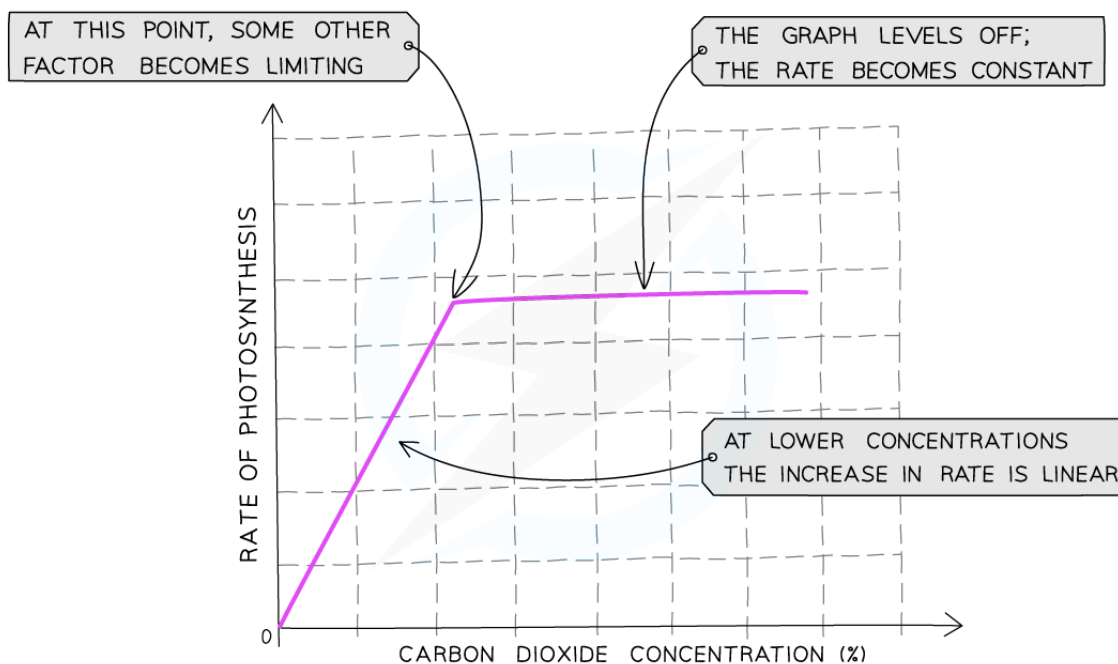
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### The effect of light intensity on the rate of photosynthesis

## Carbon dioxide concentration

- The **same basic experimental setup** can be used, but with varying use of the following variables
- Start with boiled and re-cooled water as before
- Add **successive masses of sodium hydrogencarbonate** to increase the concentration in increments of  $0.01 \text{ mol dm}^{-3}$ , and record the rate of photosynthesis in  $\text{bubbles minute}^{-1}$
- **Keep the temperature constant** at  $25^\circ\text{C}$  using a water bath, monitoring with a thermometer in the water surrounding the aquatic plant
- **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant
- A graph of the effect of carbon dioxide concentration against the rate of photosynthesis shows a similar trend to what was observed with light intensity



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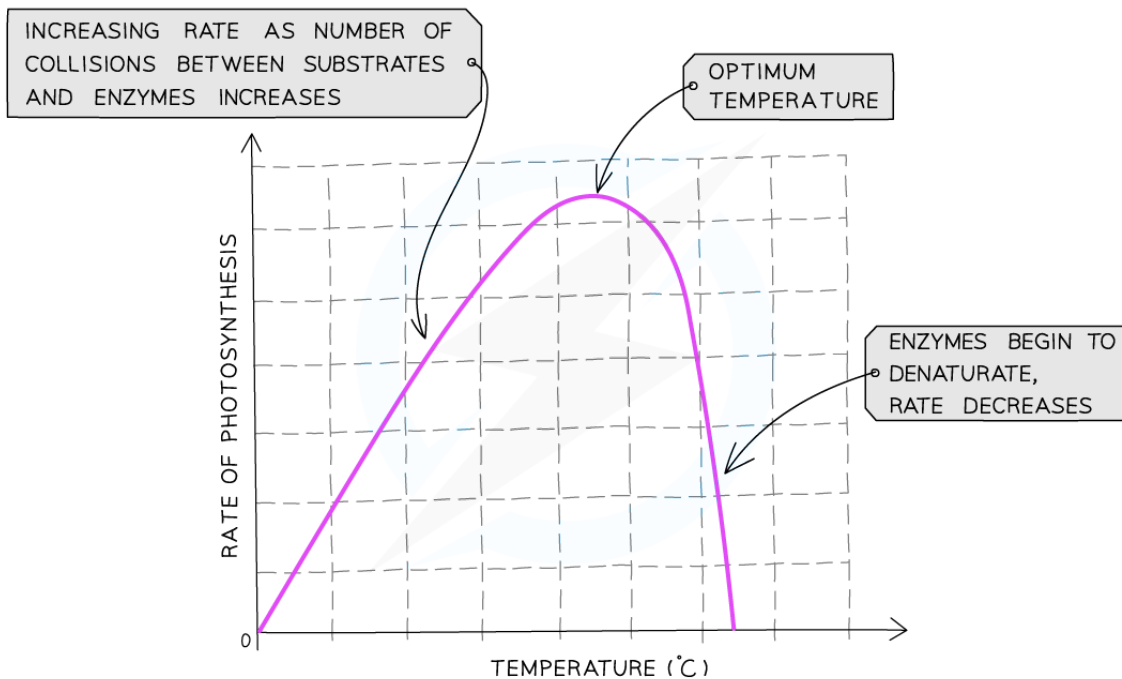
### The effect of carbon dioxide concentration on the rate of photosynthesis

## Temperature

- The **same basic experimental setup** can be used, but with varying use of the following variables
- Start with boiled and re-cooled water as before, with **sodium hydrogencarbonate at a fixed concentration** of  $0.1 \text{ mol dm}^{-3}$ , and record the rate of photosynthesis in bubbles  $\text{minute}^{-1}$
- **Vary the temperature** from  $5^\circ\text{C}$  to  $50^\circ\text{C}$  using water baths, monitoring with a thermometer in the water surrounding the plant
- **Keep the light intensity constant** by keeping the lamp a fixed distance from the plant
- It can be seen from a graph of the effect of temperature on the rate of photosynthesis that:
  - An increase in temperature will result in an increased rate of photosynthesis
    - This is due to the increase in **kinetic energy** of enzyme and substrate molecules which results in more collisions and formation of more enzyme-substrate complexes
  - This increase will continue until the **optimum temperature** for the enzyme is reached
  - Any further increase in temperature will see a **decrease** in the rate of photosynthesis
    - As enzymes begin to denature, they cannot form enzyme-substrate complexes anymore and therefore cannot catalyse the reaction



Your notes



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### The effect of temperature on the rate of photosynthesis

#### Examiner Tip

The key to this part of the spec is to appreciate how an experimental investigation can be controlled so that any effects we observe **are directly due to the one variable** that we are deliberately changing.



Your notes

## Carbon Dioxide Enrichment Experiments

### Carbon Dioxide Enrichment Experiments

- **Future rates** of photosynthesis and plant growth can be predicted using experiments such as
  - enclosed greenhouse experiments
  - free air carbon dioxide enrichment experiments (FACE)
- Due to the impact of global warming already documented and rising levels of **greenhouse gases**, including **carbon dioxide**, it is fundamental that studies are carried out to establish the effect of **carbon dioxide on plant growth and photosynthesis** to develop a clearer idea of the potential future risks that we may encounter

#### Enclosed greenhouse experiments

- Monitoring photosynthesis and growth can be done using an **enclosed greenhouse** or polytunnel set up
- This allows variables to be **manipulated** or **controlled** in order to establish the impact of different factors
- Only **small species** that can be contained in a greenhouse can be studied using this method
- **Variables** that would be manipulated might include
  - light
  - carbon dioxide
  - temperature
  - wavelengths of light
- Other variables should be controlled so as to ensure that the effect of only **one variable** is being considered at any one time

#### Free air carbon dioxide enrichment experiments (FACE)

- These experiments are carried out in natural ecosystems where carbon dioxide is pumped into the area to increase the localised carbon dioxide concentrations
- This set up allows larger plants and trees to be studied
- Other variables cannot be controlled in these scenarios but they can be monitored to establish any relationships that may become apparent in the data

#### NOS: Finding methods for careful control of variables is part of experimental design

- In an experiment, a variable is any factor that could change or be changed
  - The **independent variable**: the only variable that should be changed throughout an experiment
  - The **controlled/confounding variables**: any other variables that may affect the results of the experiment that need to be controlled or monitored
  - The **dependent variable**: the variable that is measured to determine the outcome of an experiment (the results)
- It is essential that any variable that may affect the outcome of an experiment is controlled in order for the results to be **valid**

- **Preliminary research** and preliminary studies can be used to **identify variables** within an experiment and to determine ways of controlling these variables effectively
- The science surrounding the issue/problem being investigated is likely to contain information about different factors or variables that may exist



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