Biology A2 Coursework

How temperature affects the rate of photosynthesis

Contents:

Abstract: The aim of the experiment was to observe and analyse the affect of temperature on the rate of photosynthesis. This rate was measured by the rate of oxygen produced (mm^3/min), by a photosynthesising plant (elodea), under different temperatures. A specialised apparatus called a potometer was used to observe this affect. A certain length of elodea (50mm) was cut at a slant at one end, and was placed in a test tube and was immersed in the pondweed water it was accustomed to. The cut end of the elodea was attached to a delivery tube which was connected to a measuring tube which was further attached to a syringe by rubber tubing. This whole apparatus (the potometer) was then placed in varying temperatures to measure the affect of the temperatures on the rate of oxygen production. For each temperature the length of the oxygen bubble released by the plant was measured by pulling it into the measuring tube of the potometer by using the syringe. This length was recorded and converted to the rate of oxygen produced by the plant per minute under that temperature (mm^3/min). The same elodea was used to perform the experiment under the same temperature three times all together i.e. there were three repeats for each temperature. The temperatures used were: 0 ^oC, 15 ^oC, 25^oC, 35 ^oC, 45 ^oC, 55^oC, and 65 ^oC.
Many factors affect the rate of photosynthesis mainly carbon dioxide concentration (controlling the rate of the Calvin cycle), light intensity (Affecting the light stage) and temperature (affecting the kinetic energy of all the molecules, including enzymes involved in the photosynthetic reactions and reducing the rate of photosynthesis by denaturing the enzymes after a certain temperature). Temperature was the only factor to be varied, which meant that the other two limiting actors had to be available constantly and in plenty. Excess carbon dioxide was provided by adding equal amounts of sodium hydrogen bicarbonate to each test tube which dissolved in water to provide carbon dioxide and the light intensity was controlled by providing only one artificial source of light i.e. a lamp which was kept at the same distance of 50mm from each test tube (with the elodea in it).
The shape of the graphs and the results obtained made me realise that the rate of photosynthesis mainly depends on the functioning of enzymes. The graphs showed an increase in the average rate of oxygen production between 0 ^oC -45 ^oC (with peaks forming at approximately 42 ^oC i.e. at the optimum temperature). As the temperature increased beyond 45 ^oC, the rate of oxygen production decreased steeply and the lowest rate was recorded at 65 ^oC. The t test value was calculated for 0 ^oC and 35 ^oC, 25 ^oC and 55 ^oC and 35 ^oC and 65 ^oC.
Valid conclusions were made and most of the results were in accordance with the prediction although there were some anomalies present. The errors and limitation of the experiment were evaluated and certain improvements were suggested.

Introduction: Photosynthesis (photo=light, synthesis=putting together) is defined as the trapping or fixation of carbon dioxide and its subsequent reduction to carbohydrate (sugars), using hydrogen from water. These sugars can then be converted into other essential substances like fats, proteins etc- which plants need to live and grow. *[Text adapted from OCR A2 Biology Textbook]*

Photosynthesis occurs mainly in the leaves of green plants which contain a light trapping pigment called chlorophyll. The raw materials needed for photosynthesis to occur are carbon dioxide (CO_2) which is obtained from the air via stomata by diffusion and water (H_2O) from the soil which is transported up to the leaf via the root hair and the xylem tissue. These act under the action of light energy trapped in the

chlorophyll of the leaf to form the sugar glucose ($C_6H_{12}O_6$) which is a carbohydrate and oxygen (O_2), which is released as a by product through the stomata into the atmosphere.
The general equation for photosynthesis in a green plant is:

$$\text{Carbon dioxide} + \text{water} \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} \text{glucose} + \text{oxygen}$$

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light energy}} C_6H_{12}O_6 + 6O_2$$

Photosynthesis takes place in two steps. These are light dependant reactions which require light energy and the light independent reactions, for which light energy is not needed.
Light energy drives the light dependant reactions and the products formed at the end of it account for the functioning of the light independent reactions. Light energy is trapped from the source like the sun, by two types of photosynthetic pigments:

Pigment		Colour
Chlorophylls	Chlorophyll a	Yellow-green
	Chlorophyll b	Blue-green
Carotenoids	B carotene	Orange
	Xanthophyll	Yellow

1. <u>The light-dependant reactions</u>: The light independent reactions take place in the grana of the chloroplast. These reactions include:
    - Synthesis of ATP from ADP in photophosphorylation.
    - Splitting of water by photolysis to give hydrogen ions
    - Production of reduced NADP

Photophosphorylation of ATP can be cyclic and non cyclic, which depends on the pattern of electron flow in the photosystems.
Cyclic Photophosphorylation: Light energy is absorbed by P700-photosystem I, by the accessory pigments and is transferred to the primary pigment i.e. chlorophyll a. Electrons in the chlorophyll a molecule are excited and reach a higher energy level and are emitted. This excited electron is captured by an electron carrier and channelled back to chlorophyll a molecule through a chain of electron carriers. The electrons lose energy as they pass through electron carriers. This energy is used to synthesise ATP from ADP and an inorganic phosphate group (Pi). This ATP is required in the light independent stage i.e. the Calvin cycle. The electron is sent back to photosystem 1 via electron carriers after ATP is formed, thereby completing the cycle.

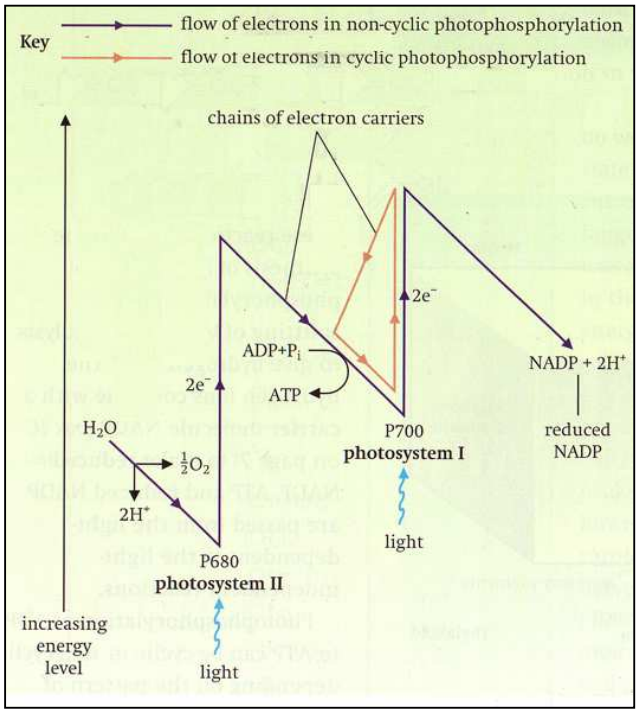
<u>Non cyclic Photophosphorylation</u>: Non cyclic photophosphorylation involves both photosystem 1 and photosystem 2. Light energy is absorbed by both photosystems and excited electrons are emitted from the primary pigments of both reaction centres (P680 and P700). These electrons are absorbed by electron acceptors and passed along electron carriers leaving the photosystems positively charged. The electrons emitted from photosystem II travel to photosystem 1 and stabilise it. *[Text adapted from Page*

20 of OCR A2 Biology Textbook] . To stabilise photosystem II, photolysis of water occurs to form hydrogen ions, oxygen and electrons are released. This reaction is catalysed by a water splitting enzyme present in the photosystem itself.

$$H_2O \longrightarrow 2H^+ + 2e^- + \tfrac{1}{2}O_2$$

These electrons produced during the photolysis of water are absorbed by P680 and stabilise it. The hydrogen ions combine with the electrons released from photosystem I (P700) and a carrier molecule called NADP to from reduced NADP. This reduced NADP is of importance in the Calvin cycle or the dark stage. As in cyclic photophosphorylation ATP is synthesised as electrons lose energy while passing along the electron carriers throughout. The formation of reduced NADP is shown by the following equation:

$$2H^+ + 2e^- + NADP \longrightarrow \text{Reduced NADP (NADPH)}$$



[Diagram taken from [1] http://content.answers.com/main/content/wp/en/6/6d/Z-Scheme.PNG]

As photolysis is carried under the action of a water splitting enzyme, temperature would have an effect on the rate of splitting up of water and therefore the rate at which electrons are released i.e. rate at which photolysis takes place. This is because the action of enzymes depends on the temperature as a low temperature would reduce there action (low kinetic energy) whereas a high temperature of about 40 degrees would increase the rate at which they carry out their processes (high kinetic energy). But a temperature higher than 40 degrees can denature them, and the enzymes can stop functioning.

Oxygen is the only measurable product of photolysis, and the release of oxygen depends on the enzyme controlled photolysis of water. So temperature affects the functioning of enzymes and therefore the rate at which oxygen is produced during the light stage of photosynthesis. The rate at which oxygen is evolved in a fixed amount

of time can be used to measure the rate of photosynthesis in the experiment under varying temperatures.
Light intensity will also have an affect on how fast the products of light dependant stage are formed. A high light intensity will mean more energy available for the electrons to be excited, so both cyclic and non cyclic photophosphorylation will take place at a higher rate. An increase in the rate of cyclic photophosphorylation will mean more ATP will be transported to the dark stage to be used. So the dark stage will also occur at a higher rate increasing the overall rate of photosynthesis. Because of the high rate of reaction of the non cyclic photophosphorylation, photolysis of water will occur at a higher rate and more oxygen will be produced. A low light intensity can reduce the amount of oxygen released because the electrons will be less excited and both the cyclic and non cyclic photophosphorylation will take place at a lower rate. Again photolysis of water will be slower and less oxygen will be produced. Also less ATP and reduced NADP (produced in the non cyclic photophosphorylation) will be available for the dark stage to use and this in turn will reduce the rate at which the dark stage produces its products. This would reduce the overall rate of photosynthesis and so light intensity will have to be considered during the experiment.

2. <u>The light independent reactions of photosynthesis</u>: This stage is known as the Calvin cycle or the Dark stage as light is not required here and it takes place in the stoma region of the chloroplast. Carbon dioxide from the atmosphere is the raw material required to carry out these reactions. The reactions that take place are:

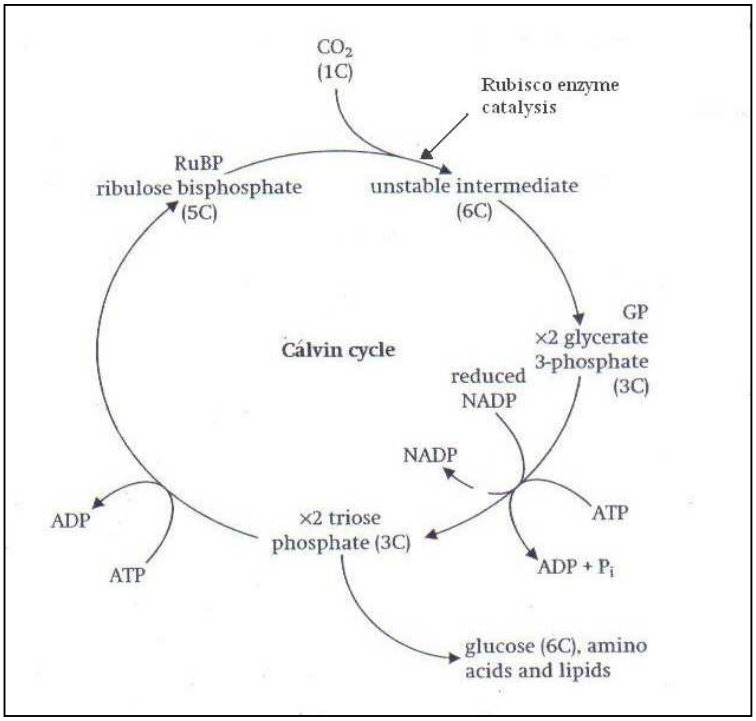
[The following bullet points are adapted from page 21-22 of OCR A2 Biology]

- The first stage is the fixation of carbon dioxide called carboxylation. The carbon dioxide combines with a 5 carbon sugar- Ribulose Bisphosphate (RuBP) under the action of an enzyme called Ribulose Bisphosphate Carboxylase (Rubisco). The result is an unstable intermediate of 6 carbons.
- This intermediate breaks down to form two molecules of a three carbon compound named Glycerate 3- phosphate (GP or PGA). Then GP is reduced to two molecules of a three carbon sugar called triose phosphate (TP) by using the energy from ATP and the hydrogen atom from the reduced NADP, both received from the light dependant stages of photosynthesis. As ATP is used, ADP and Pi are released and as the hydrogen atom from reduced NADP is used, NADP is released. ADP, Pi and NADP are returned to the light dependant stage to further from ATP and NADP respectively to be used in the Calvin cycle again.
- Some of the triose phosphates condense to form six carbon sugars like sucrose, starch and cellulose or are converted to acetylcoenzyme A which forms amino acids and lipids. Other molecules of triose phosphate are used to regenerate Rubilose Bisphosphate (RuBP) which again combines with carbon dioxide under the action of Rubisco and the process starts again. The regeneration of RuBP also requires ATP, at the end of which ADP is reformed to be used in the light stage again.

At the end of the light independent reactions, carbohydrates (products of photosynthesis) are formed. The most important raw material for these reactions is carbon dioxide. Therefore the availability of carbon dioxide has a direct relation to the rate at which photosynthesis takes place. So during the experiment, the

concentration of carbon dioxide should be controlled to make the test fair. Therefore external supplies of carbon dioxide should be provided like Sodium hydrogen carbonate ($NaHCO_3$) which reacts with water to form carbon dioxide. This would make it available in excess for the plant to use. Also carboxylation requires the action of an enzyme (Rubisco). Again temperature will have a direct affect on the functioning of the enzyme Rubisco and therefore the rate at which photosynthesis occurs.

Diagram showing the Calvin Cycle



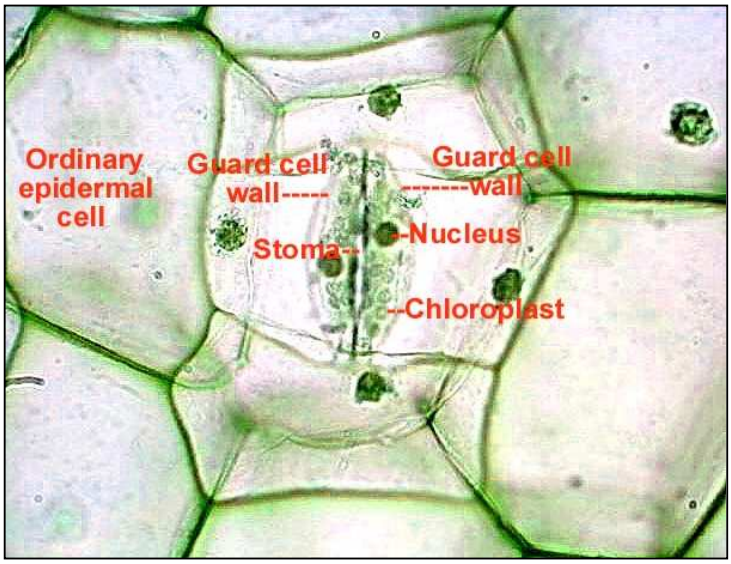
[Diagram taken from Page 21 - OCR A2 Biology]

Light intensity has an indirect affect on the reactions taking place in the light independent stage. As mentioned above, if the light intensity is low, then the electrons in the light dependant stage will be less excited. Therefore the rates of cyclic and non cyclic photophosphorylation will be low. This will result in less ATP and reduced NADP being formed from both phases of photophosphorylation. As these products are the key to the functioning of the light independent stage, a shortage can result in a lower rate of reactions in the Calvin cycle, thereby producing low amount of products like triose phosphate and to regenerate Ribulose Bisphosphate. This can result in an overall reduction in the rate of photosynthesis. So light intensity is a very important factor in the deciding the rate of photosynthesis and will have to be considered during the experiment.

Guard cell*s*: As photosynthesis takes place, carbon dioxide enters the plant (i.e. into the leaf) and oxygen leaves the plant (as a by product of photolysis of water). This gaseous exchange occurs through tiny pores present in the lower epidermis of the leaf called the stomata. These stomata also provide a gateway for water to move in and out

during transpiration. Each stoma (singular) is bounded by two guard cells which regulate the opening and closing of the stomata. The wall of the guard cells next to the stoma is very thick and the wall furthest from the pore is very thin. When water moves into the guard cells, the thin wall bends more readily than the thick wall and this makes the guard cells turgid. The guard cells become curved and the stomata open. The reverse happens when water moves out of the guard cells and they become flaccid, thereby closing the stomata not allowing any gaseous exchange and transpiration to take place.

A diagram showing guard cells surrounding a stomata pore:



[*Diagram taken from http://www.lima.ohio-state.edu/academics/biology/images/stoma.jpg*]

Gaseous exchange depends on the opening and closing of the stomata which is controlled by the turgidity of the guard cells. In the experiment the amount of oxygen released will be measured, which moves out of the leaves via the stomata. During very high temperatures, the guard cells close due to loss of water by evaporation and the stomata are blocked, preventing any gaseous exchange to take place. Therefore no oxygen is allowed out of the leaf. Also no carbon dioxide will be allowed to enter the leaf and the reactions taking place in the Calvin cycle will cease or their rate will decrease. This affect of temperature in reducing the rate of photosynthesis can be considered while drawing conclusions during the experiment.

Enzymes: Enzymes are globular proteins and act as biological catalysts. Being catalysts, enzymes increase the rate of a particular reaction without being chemically altered themselves. “In the body, they increase the rate of reactions by a factor of between 10^6 to 10^{12} times, allowing the chemical reactions that make life possible to take place at normal temperatures”.
[Data adapted from http://www.biologymad.com/master.html?http://www.biologymad.com/Enzymes/enzymes.htm]

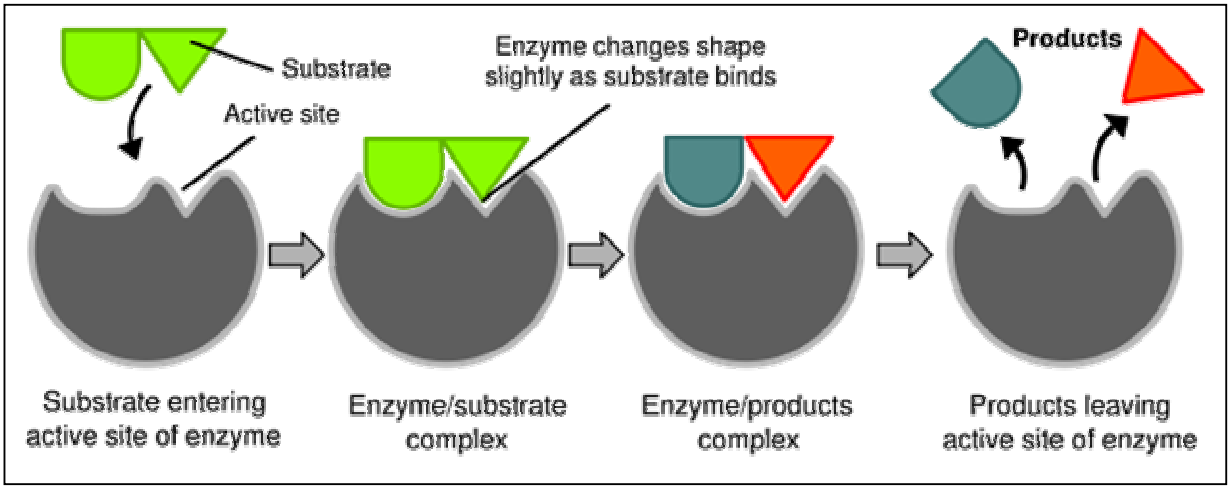
Some stages of photosynthesis occur under the action of enzymes, like ribulose bisphosphate carboxylase (Rubisco), the water splitting enzyme for photolysis and ATP synthase for the synthesis of ATP.

As enzymes are proteins, they also have a primary, secondary and tertiary structure. The primary structure consists of the sequence of the amino acids joined together by peptide bonds to form a polypeptide chain. One or more polypeptide chains formed by the primary structure, coil into an alpha helix or a beta pleated sheets. This occurs due to the hydrogen bonding between the different amino acids present in the chain. The tertiary structure is formed when the secondary structure of the protein (enzyme in this case) is coiled and folded up to from a precise 3 dimensional shape. This shape of the molecule is very precise and is held in place by various bonds between the amino acids at different parts of the coiled polypeptide chains. These bonds include hydrogen bonds, disulphide bonds, ionic bonds and the hyd rophobic bonds (or van der walls forces). *[This text is adapted from OCR AS biology text book]* .

Each different enzyme has its own highly specific shape with a depression. This depression is called the active site. The active site for all molecules of one enzyme will be made up of the same arrangement of amino acids; it has a highly specific shape. The active site is where the substrate i.e. the reactant molecule binds with the enzyme molecule. The product is called an enzyme-substrate complex. This 3 dimensional structure of enzymes is determined by the sequence of amino acids present in the tertiary structure of each molecule.

The active site of each enzyme molecule has is complementary to an individual substrate molecule. So only one type of substrate molecule can bind to the active site of a particular type of enzyme. In other words, each active site of an enzyme is specific to a substrate molecule. When the substrate molecule binds to the active site, temporary bonds are formed between the substrate and some of the enzyme's amino acids and a complex is formed. Structural changes occur so that the active site fits precisely around the substrate. This mechanism is referred to as the "induced fit" mechanism as the substrate induces the active site to change shape.

A diagram showing the "induced fit" mechanism:
[Diagram adapted from http://en.wikipedia.org/wiki/Image:Induced_fit_diagram.svg]



The reaction will take place and the product, being a different shape to the substrate, moves away from the active site. The active site then returns to its original shape and is free to react with another substrate molecule. During this reaction, bonds can be broken or be made. So the reactant molecules i.e. substrate can be one single molecule which can be broken down into two or more products, or it can be two or more molecules which bind to the active site and are formed into one product.

An enzyme speeds up a reaction by lowering the minimum amount of energy required to form the unstable intermediate known as the transition state which is a "hybrid" structure between the reactants and the product (the enzyme substrate complex). This energy is called the activation energy. The larger the activation energy, the slower the rate of reaction only a few substrate molecules will, by chance, have sufficient energy to overcome the activation energy barrier. When a substrate molecule binds to the enzyme, the active site changes shape and fits itself around the molecule, distorting it into forming the transition state, and so speeding up the reaction. This is referred to as the induced fit mechanism. So enzymes lower the activation energy by stabilising the transition state, and they do this by changing the reaction conditions within the active site of the enzyme. *[The above text is adapted from http://www.biologymad.com/master.html?http://www.biologymad.com/Enzymes/enzymes.htm]*

The way a substrate molecule binds to an active site is completely by chance. The collisions between the enzymes and substrate create this chance of a reaction to take place and more the number of successful collisions, faster the rate of reaction. The probability of successful collisions can only be created by increasing the number of collisions. The probability of the enzymes and substrate colliding and therefore increasing the rate of the reaction, can be controlled by increasing the quantity of enzyme molecules (therefore number of active sites) or increasing the number of substrate molecules, or increasing the speed at which the enzyme and substrate molecules move within the reaction site. "As the speed of the molecules increases, they gain more kinetic energy and collide more often. Moreover they do so with more energy, enough to break or make the bonds and to form products." *[Text adapted from AS Biology for OCR]*. So the rate at which a substrate molecule binds to an active site determines the rate of a reaction and this can only be controlled by high number of successful collisions.

Temperature has an effect on the working of enzymes and therefore it also controls the rate of reaction under certain conditions. A moving substance always has kinetic energy and temperature can be responsible for an increase or decrease in kinetic energy. At a low temperature, an enzyme and a substrate molecule will have a low kinetic energy and will be moving slowly. But as temperature increases, both the enzyme and substrate molecules start to vibrate and gain more kinetic energy. This means that they move faster around the environment they are present in and are more likely to collide with each other. This increases the chance of successful collisions i.e. a substrate molecule binding to the active site and reaction occurring. However an increase in the rate of reaction only happens until a certain temperature. Enzymes have a temperature where they work the fastest; this is called the optimum temperature. An increase in temperature makes the enzyme molecules gain more kinetic energy as they vibrate more and this increases the number of collisions and results in an increase in the rate of reaction. But if the enzymes gain a very large

amount of kinetic energy, they being to vibrate uncontrollably and the bonds holding the tertiary structure together are weakened or they break. This causes the specific active site of the enzyme molecule to be distorted and the enzyme is said to be denatured. So the active site of the enzyme is no longer complementary to the substrate molecule and it doesn't fit the active site no more. The enzyme is said to be denatured. Therefore the enzyme does not bind to the substrate molecule and does not catalyse the reaction and the rate of reaction is decreased. So temperature increases the rate of reactions controlled by enzymes but only until the optimum temperature of the enzymes is reached. The optimum temperature of enzymes to work is about 37.5^{o}C.

The experiment will investigate the affect of temperature on the rate of photosynthesis and temperature affects some important stages of photosynthesis like the Calvin cycle controlled by Rubisco. A high temperature accounts for the high rate of reaction at those stages, but a very high temperature (>40 C), will denature the enzymes and the reactions occurring at that particular stage will decrease or the reactions will no longer take place. This would result in less or no product being formed and the rate of photosynthesis to decrease.

Other factors affect the functioning of enzymes apart from temperature. Firstly an increase in the concentration of substrate molecules affects enzyme actions as it increases the probability of an enzyme colliding with a substrate molecule and forming an enzyme substrate complex. A decrease in substrate concentration will have the reverse affect of decreasing the probability of an enzyme substrate complex to be formed. pH can cause enzyme molecules to denature by disrupting the specific 3 dimensional shape of the enzyme molecule. Different substrate molecules called inhibitors have shapes similar to the active site of the enzyme molecules and can block the active site, thereby making the enzyme ineffective. All these factors including temperature have an impact on how the enzymes work and therefore the rate of the reactions controlled by the enzyme.

Therefore, there are many factors which increase or decrease the rate of photosynthesis.
Light energy is needed during the light stage of photosynthesis to excite electrons. More light would mean higher rate of reaction as more electrons would be excited and less electrons would be excited during low light intensity.
Light energy is also the most important external factor as it is converted to chemical energy during photosynthesis. As mentioned above, light intensity has a very significant affect on the rates at which products of photosynthesis are produced by being a raw material for the light dependant and indirectly affecting the light independent stages of photosynthesis.

Carbon dioxide is responsible for Calvin cycle to take place and therefore for glucose to be formed. So high carbon dioxide concentration would mean a faster rate at which glucose is formed and so a higher rate of photosynthesis and a low amount of carbon dioxide would mean less glucose and therefore slower rate of photosynthesis.

Water is needed during photolysis to provide the oxygen which is released as a waste product, but during the experiment the amount of oxygen released will determine the rate at which photosynthesis is taking place. Therefore if more water is available for

photolysis, a higher amount of oxygen will be released and a higher the rate of photosynthesis will be recorded. Water concentration also affects the turgidity of the guard cells, and if present in a short supply can close the stomata pores and disallow any or a low amount of carbon dioxide form entering the plant and restricting oxygen to be released out of the plant.

But the most important limiting factor which alters the rate of reaction is the temperature. A high temperature increases the rate of reaction by providing the molecules with a higher amount of kinetic energy which results in more collisions with sufficient energy to break or make bonds. More products are formed and the rate of reaction is increased. Temperature also controls the rate of reactions by having an affect on enzymes. A high temperature will again result in higher number of collisions and therefore a larger amount of enzyme substrate complex to be formed. But a temperature higher than the optimum temperature of the enzymes will result in the enzymes being denatured as their specific 3 Dimensional shape is altered and the substrate is no longer complementary to the changed active site. This accounts for a decrease in the number of products formed and therefore a decrease in the rate of reaction. During photosynthesis the light independent stage depends on temperature. Moreover factors like enzyme and substrate concentration, and presence of any inhibitors also affects the rate of photosynthesis.

All these factors will be taken into account while performing the experiment and to provide conclusions from the results that will be obtained.

Preliminary Experiment:

Aim: To investigate how temperature (of water) affects the number of oxygen bubbles produced by elodea.

Apparatus:
Test Tube (x4)
Beaker(x4)
Test tube stand (x1)
Elodea (pond weed)
Water
Thermometer (x1)
Kettle
Scissors
Ruler
Stop Clock
Lamp (x1)

Prediction: I predict that as the temperature increases from 10 ^{o}C to 30 ^{o}C, so will the rate of photosynthesis. But at a temperature higher than 30 ^{o}C i.e. 50 ^{o}C and 70 ^{o}C, I predict that the rate of photosynthesis will decrease. I also predict that the bubbles produced at 30 ^{o}C would be the highest and at 70 ^{o}C will be the lowest.

Method:
1. Collect all equipment required.
2. Prepare 4 equal lengths of elodea each 50mm each using a ruler and scissors.

3. Fill 4 test tubes with tap water enough to immerse the 50mm elodea completely.
4. Using a kettle prepare water of temperature 70 °C and fill a beaker with the prepared water halfway.
5. Make sure the temperature is 70 °C using a thermometer and use ice if needed to be cooled.
6. Place the test tube in the beaker.
7. Place the lamp 50mm away from the beaker and switch it on.
8. Using the stop clock, let photosynthesis take place for 5 minutes.
9. During the 5minutes, count the total number of bubbles produced by the elodea and note down the results.
10. Repeat steps 4-8 for 10 °C, 30 C and 50 °C
11. Prepare a table of results.

Results:

	Number of bubbles released after 5 minutes
70 °C	7
50 °C	12
30 °C	240
10 °C	18

Conclusion:

At the lowest temperature (10 °C), the number of bubbles produced were low as expected which showed that the rate of photosynthesis was slow. As the temperature increased to 30 °C the number of bubbles released increased to 240 which showed that the rate of photosynthesis increased. This result was also the highest compared to all other temperatures which again proved the prediction right. Also as temperature increased, so did the rate of photosynthesis. At 50 °C the number of bubbles released decreased to 12 bubbles and at 70 °C the number of bubbles were 7 only, which was the lowest result recorded. This was also expected and as the temperature increased above 30 °C, rate of photosynthesis (number of bubbles) decreased.

Evaluation:

The preliminary experiment was suitable to gain a general idea of the effect of temperature on the rate of photosynthesis but many changes have to be made in the actual experiment. These are:

- Although using scissors to cut the elodea to 50mm length was a pretty accurate, but in the actual experiment a scalpel can be used to cut the elodea as a scalpel will cut the elodea more easily and more accurately. Also the elodea has to be cut at an angle to prevent any air bubbles from entering the plant and this would be easily done with a scalpel rather than a pair of scissors.
- Filling the test tubes with tap water did fulfil the condition of using the water from the same source to maintain the water potential of the external environment, but no time was given to let the elodea plant to acclimatise. So in the actual experiment the elodea can be let to acclimatise in the test tube still using water from the same source. But this can be time consuming. So the elodea to be used can already be kept overnight in water, and the same water in which the elodea was kept can be used in the test tube to immerse the elodea during the experiment. This would make the elodea adjust to the water

environment by making conditions suitable for it to survive. The elodea will also acclimatise to the temperature of the water and will not have to reacclimatise if the surrounding water in unchanged. Also it would already be performing photosynthesis and therefore significant changes in results can be noted.

- In the actual experiment, the elodea would be subjected to the lamp after it has been left to acclimatise in water and a different lighted source overnight. As mentioned earlier, the elodea would be immersed in the same water as in which it was kept overnight. To ensure that the plant acclimatises to the new light source, 2 minutes will be allowed before timing the experiment, to let the elodea adapt to the light intensity of the lamp and the photosynthesis to initiate. Then the clock will be started and the rate will be measured.
- To gain higher temperatures like 30 ^{o}C, 50 ^{o}C and 70 ^{o}C, a kettle was used. This method can be improved upon by using a more accurate way to keep the temperature constant i.e. using water baths for higher temperatures. A big enough water bath can be used to carry out the initial experiment and the repeats in the same bath unlike using a kettle which would require reheating the water again and again if more water is needed for the same temperature. Also water can only be heated till a single temperature in a kettle and to produce lower temperatures, cold water will have to be added. This isn't a very accurate way of measuring temperature and not very reliable because it would be very hard to produce the same temperature of the water again.
- For the preliminary experiment, although the same lamp was used for each temperature which ensured the light intensity was the same, no effort was made to make the lamp the only source of light intensity. For the actual experiment, all other sources of light have to be covered or kept constant for all repeats and temperatures. These would include covering up windows with black paper and switching off all lights in the experiment room. Also as light would be in abundance, it would not be a limiting factor, which would ensure that the results obtained would be because of change in temperature.
- Carbon dioxide concentration also has an affect on the rate of photosynthesis. This is one of the factors which cannot be controlled, but to ensure fair test equal amounts of carbon dioxide can be added to each test tube before exposing it to a light source in the actual experiment. This would be done by adding the same amount of Sodium Hydroxide ($NaHCO_3$) to each test tube. The sodium hydroxide will provide excess carbon dioxide to each elodea plant so that carbon dioxide is eliminated from being a limiting factor as photosynthesis is occurring.
- The way of counting the number of bubbles to measure the rate of photosynthesis was not very accurate because the bubbles were of different sizes and some of the bubbles could have been missed out due to human error. So for the final experiment, a potometer will be used to measure the length of all the bubbles would be measured by calculating the volume of gas released. This would give a more valid result.
- The elodea pieces were taken from different pondweed plants. This meant that they had different rates at which they released oxygen as they could have had varying number of leaves for the same 50mm of elodea. Difference in oxygen production could also have been caused by the plants being kept in different environments which could have affected the health of the plant. To make sure only temperature affects the results, the lengths of elodea will be cut from the

same plant during the final experiment at least for each temperature and its repeats.

- Only four temperatures were used for the preliminary experiment and the temperatures were 20 ^{o}C apart. For the final investigation, 7 different temperatures would be used and the range would be much closer. The temperatures that would be used are- 0 ^{o}C, 15 ^{o}C, 25 ^{o}C, 35 ^{o}C, 45 ^{o}C, 55 ^{o}C and 65 oC. Also no repeats were made for any of the temperature during the preliminary work, so for the final experiment two repeats would be made for each temperature so that the average of the results can be calculated giving more accurate and reliable results.

Also precautions will be taken to make sure that all the apparatus are clean before starting the experiment so that no traces of water or any other substance is present which might affect the results in any way.

Aim: To investigate the affect of temperature on the rate oh photosynthesis

Prediction:
As temperature is the factor to be controlled, I predict that as temperature increases, so will the rate at which photosynthesis takes place.

The light dependent stage is dependant on light energy to excite electrons in the cyclic and non cyclic photophosphorylation. The light independent stage depends on temperature as the energy source and it has a direct affect on the rate at which the dark stage occurs. As temperature will be increased, all the molecules of the dark stage will gain more kinetic energy and the probability of them colliding and forming or breaking bonds will increase. Therefore more products will be formed and the rate of the reactions will increase. Increase in temperature can also cause the molecules in the light stage to gain an increase in kinetic energy and to react faster. An indirect affect on temperature in increasing or decreasing the rate of photosynthesis is its affect on enzyme activity. The light dependant stage is mostly dependant on light as an energy source whereas the light independent stage doesn't depend on light as the source, but on the products of light dependant stage i.e., ATP and reduced NADP. But temperature plays an important part in both the stages. These are the two most important reactions as they produce the products of photosynthesis- oxygen and glucose respectively. Photolysis of water is an enzyme controlled reaction and takes place in the light dependant stage. The dark stage is controlled by Ribulose Bisphosphate carboxylase (Rubisco) which catalyses the fixation of carbon dioxide with Ribulose Bisphosphate (RuBP). These enzymes have an active site complementary to the reactant molecules (Water in the light stage and, RuBP and carbon dioxide in the dark stage). At a temperature of 0 ^{o}C which is the lowest temperature to be used in the experiment, the enzyme and substrate molecules have very low kinetic energy. Due to their slow movement, the numbers of collisions are low and therefore not enough water molecules or carbon dioxide and RuBP molecules that bind with their respective specific enzymes to form the enzyme substrate complex i.e. the product. Therefore fewer products are formed as the rate of reaction decreases i.e. less oxygen and hydrogen ions are produced during photolysis and less unstable intermediate and eventually less glucose is formed at the dark stage. Therefore the overall rate of photosynthesis is low. Although the reactions are being catalysed, but these reactions are occurring at a very low rate. The activation energy required for the reaction is decreased, but is not decreased by a great amount and still a high amount of energy is required to reach the transition stage and to from the enzyme substrate complex.

As the temperature increases, the enzymes gain more kinetic energy and vibrate more rapidly. This increases the number of successful collisions as the likelihood of the molecules binding to the active site and reacting is increased. So, as more kinetic energy is gained, more substrate molecules combine with their complementary enzymes and more of them are held in a convenient position in the active site. The enzyme changes shape to make the substrate molecule(s), from or break bonds as quickly as possible. This reduces the activation energy and less energy is required to reach the transition stage. As more products are formed by the reactions controlled by enzymes like photolysis and carbon fixation, the rate of photosynthesis is quicker. So higher the increase in temperature, higher the amount of kinetic energy gained by the

enzyme and substrate molecules, higher the probability of successful collisions and therefore faster the rate of photosynthesis. So the amount of oxygen and hydrogen ions produced at photolysis will increase and more unstable intermediate and therefore more glucose will be formed as a result of a higher rate of carbon fixation at the dark stage. In the experiment, as the temperature will increase, a large length of the oxygen bubble will be recorded.

As the temperature increases from 30 ^{o}C to around 38 ^{o}C, the rate at which photosynthesis is taking place will keep increasing constantly with it being the lowest at 30 ^{o}C and highest at 38 ^{o}C. This means that the kinetic energy gained enzyme and substrate molecules involved in the process of photosynthesis, will increase as the temperature rises from 30 ^{o}C to 38 ^{o}C, and the number of successful collision will also increase in proportion to temperature. The amount of product produced too will increase as the temperature reaches 38 ^{o}C. So, at approximately 38 ^{o}C the highest amount of oxygen would be released i.e. the largest length of air bubble would be measured as the rate of photosynthesis would be highest at this or around this temperature owing to the high amount of kinetic energy and increase in number of collisions between enzyme and substrate molecules. This temperature where the rate of photosynthesis is the highest is called the optimum temperature and is approximately 37.5 ^{o}C.

In theory, as the temperature increases beyond 37.5 ^{o}C, the rate of photosynthesis will no longer increase. A higher temperature will have a reverse affect on the rate of photosynthesis i.e. the length of the air bubble will decrease and therefore the volume of gas released will decrease too. This is because at a temperature higher than the optimum temperature of the enzymes, the molecules gain so much kinetic energy that it causes excessive vibrations within the molecule itself. This makes the enzyme lose its specific 3 dimensional tertiary structure as the bonds holding it together break. The enzyme is said to be denatured. So the shape of the active site on the enzyme changes and is no longer complementary to the substrate molecule. Therefore, substrate molecule doesn't fit the active site and no enzyme-substrate complex is formed. This would result in the enzyme controlled reactions of photosynthesis like photolysis and carbon fixation, to take place at a lower rate or to eventually stop. The amount of products released will decrease and so would the rate of photosynthesis. For instance, during carboxylation at a temperature of 65 ^{o}C, the enzyme Rubisco will be vibrating rapidly and will eventually lose its 3D shape and the active site would be ruptured so that substrate doesn't fit into it (the substrate can be carbon dioxide molecules or RuBP molecules or both). As the reactants do not fit the active site, very few or no intermediate (enzyme substrate complex) or Glycerate phosphate (GP) or any other products of the Calvin cycle are formed and the overall rate of photosynthesis decreases as the enzymes are denatured.

Therefore generally I predict that:
As the temperature increases from 0 ^{o}C to 35 ^{o}C, the rate of photosynthesis will increase proportionally (directly proportional to increase in temperature) as there would be more number of successful collisions as temperature increases. The volume of oxygen released will be the highest at 35 ^{o}C and lowest at 0 ^{o}C.
The optimum temperature i.e. the temperature at which the rate of photosynthesis is the highest is predicted between 35-45 ^{o}C. So the largest air bubble would be measured between these temperatures.

On the other hand from temperatures between 45 ^{o}C and 65 ^{o}C, the rate of photosynthesis will decrease with it being the highest at 45 ^{o}C and lowest at 65 ^{o}C (inversely proportional to the increase in temperature). This would be because at temperature higher than 35 ^{o}C, the enzymes controlling the process of photosynthesis will denature and cease from catalysing the while reaction. Therefore the length of the oxygen bubble at 65 ^{o}C would be the smallest.
It can also be said that the two graphs which would be plotted, would be symmetrical in shape forming peaks between 35-45 ^{o}C.

Apparatus:

Apparatus	Quantity	Why were they needed
Elodea (50mm in length)	15	To provide the photosynthetic plant needed to carry out photosynthesis and from the product- oxygen which will be measured. A high amount is needed because 50mm of the plant is need for each repeat. In theory, there would be 21 experiments in all and same elodea would be needed for each repeat. So at least 7 pieces of elodea 50mm each would be needed.
Test tube	7	To put elodea in and to protect it from being in contact with very high or very low temperatures directly. 7 test tubes will be needed for the 7 different temperatures, and the same test tube will be used for each repeat.
Elodea water	200 cm^3	To provide water for the elodea to be kept inside the test tube. Elodea water will be used so that the elodea plant doesn't have to acclimatise to a new environment while the experiment is being conducted.
Thermometer	1	To measure the temperature of the water being used in the experiment and to make sure it is accurate. The thermometer being used will be accurate to 1 ^oC.
Scalpel	1	To cut the elodea to length of 50mm at an angle.
Ruler	1	To measure the length of the elodea to be cut accurate to 50mm. It will also be used to keep the lamp 50mm away from the apparatus. The ruler being used would be accurate to 1mm in length.
Water bath	5	Water bath will be used to provide the high temperatures of 35 ^oC, 45 ^oC, 55 ^oC and 65 ^oC and to keep them constant throughout the investigation.
Water	As needed	To fill the water bath so that it can be heated and the change in temperature can occur.
Potometer	1	To measure the amount of volume of gas released by measuring the length of the bubble accurately. The measuring tube attached to the potometer is 70mm long and therefore accommodate the maximum air bubble of length 70mm.
Syringe	1	To draw water into the measuring tube of the potometer so that the length of the air bubbles can be measured. The syringe to be used will be correct to 1cm^3 in volume.)
Lamp	1	To act as a source of light for photosynthesis to take place. The same lamp will be used throughout the experiment to keep the light intensity constant.
Sodium Hydrogen Carbonate ($NaHCO_3$)	30g	To act as a source of carbon dioxide and would be put in each test tube to ensure fair test and to rule out the availability of carbon dioxide being a limiting factor. 30g should be enough to be sued equally for each repeat.
Test tube rack	1	To hold the test tubes being used in the experiment while they are empty or inside the water bath.

Clamps	7	To hold the potometer connected to the plant and to hold the test tube with the elodea in it, in position to let photosynthesis to occur and to let the results to be noted clearly.
Ice	As needed	To create a low temperature of 0 ^{o}C and enough ice should be available to maintain the constant temperature.
Hammer	1	To break the size into small pieces that will be suitable for the experiment.
Tweezers	1	To handle the elodea and to prevent contact with hands this might damage the plant thereby affecting the rate of photosynthesis.
Stop clock	1	To measure accurately the time given to the plant to acclimatise and the time given to carry out photosynthesis. The stop clock will be correct to 0.01 seconds.
Plastic Tubing	1	To connect the elodea to the potometer so that the air bubbles travel to the potometer. Care should be taken to make it air tight so that no bubbles escape the tubing.
Light Intensity meter Key: A- Low light intensity H- High light intensity	1	To measure the light intensity of each apparatus set up. This would be done to record the amount of light available to the elodea and efforts would be made to keep it constant i.e. the only source of light intensity should be the lamp being used. This would ensure fair test as the factor staying constant-light, will not limit the functioning of the elodea.

Diagram:

Method:

1. Let all of the elodea acclimatise overnight in front of a lighted source and in enough water to be used for the whole experiment. *(The light source and the water will make sure that photosynthesis takes place the same water will be used in the actual experiment as already the elodea will be accustomed to conditions like the temperature making conditions suitable for its survival. Also the water potential gradient between the plant and the water will be set and so will the turgidity of the guard cells and these would remain same throughout the experiment.)*
2. Cover all the sources of light in the experiment room by covering windows with black paper or switching of any source of artificial light. *(This will ensure that the lamp being used is the only light source and would eliminate the risk of light intensity being the limiting factor.)*
3. Collect all the apparatus needed.
4. Clamp the potometer to a clamp stand and make sure the reading can be read accurately and with ease. Use another clamp stand for the syringe if needed to hold in a place convenient enough to be pulled in and out *(If the potometer isn't securely clamped it can make it hard to pull the water into the syringe and the reading can be hard to note down. Also the measurement tube has to be in a position from which the reading can be read easily even if the light from the lamp is falling on it.)*
5. Make sure the syringe is squeezed in. (*This has to be done because if the syringe is pulled out, there would already be some air trapped inside the syringe affecting the amount of water drawn into the syringe.)*
6. Cut an elodea to 50mm length using a scalpel to cut at an angle and a ruler to measure the length of the plant. Place it inside a test tube with water enough to immerse the elodea and make sure the water is the same water in which the elodea was kept overnight. Make sure the elodea is cut underwater. *(Elodea has to be immersed into the water so that no air bubbles enter the tubing and affect the volume of gas released. Elodea should be cut underwater to make sure no air bubbles are formed.)*
7. Clamp the test tube with the elodea securely to a clamp stand and connect the plastic tube to cut end of the elodea and make sure its secure but taking precaution that the elodea doesn't break. *(The tube will transport the volume of gas released which would be collected as bubbles towards the potometer. See next step. If the elodea breaks, then this again would be unfair because only the number of leaves connected to the cut end of the elodea will photosynthesise and if no leaves are present near the cut end then no bubbles will be produced.)*
8. Pull the syringe back slowly and draw water into the potometer until a constant line of water is formed. No air bubbles should be present and the cut end of the elodea should be always under water. Draw water until some of it enters the syringe. (*No air bubbles should be present because when the gas is drawn into the syringe, the air bubbles can distort the smooth line of the gas and make it hard to get an accurate measurement. To ensure no air bubbles are produced at the cut end, it has to be kept immersed under water.)*
9. Add 1g of Sodium hydrogen carbonate ($NaHCO_3$) to ensure there is enough carbon dioxide for photosynthesis to occur. (*The same amount of sodium hydrogen carbonate should be added to ensure fair test as the same amount of*

carbon dioxide will be released in all the test tubes and to eliminate it being as a limiting factor.)

10. Add the apparatus to a beaker with water (ice) at 0 ^{o}C and make sure the test tube with the elodea is immersed at least halfway into the beaker. Check the temperature is at 0 ^{o}C before inserting the test tube using a thermometer, altering the temperature as needed (*Making the test tube at least half way in contact with the ice will make sure that the surrounding temperature of the elodea water reaches 0 ^{o}C and the results can be more accurate.)*
11. Place a lamp at 50mm away from the beaker by measuring the distance using a ruler. Switch it on. Throughout out the experiment keep measuring the light intensity using a light intensity meter. (*Keeping the lamp 50mm away for each experiment will make sure that it is not a limiting factor as it is constan t. By measuring the light intensity throughout the experiment, records can be made when the light intensity changed and might have had an affect on the rate of photosynthesis.)*
12. *A*llow the Elodea to acclimatise to for 2 minutes. (Allowing acclimatising time to the elodea would ensure that the elodea gets accustomed to the new light source and the new surrounding temperature.)
13. Now let the elodea photosynthesise for 5minutes and measure the time accurately using a stop clock.
14. After 5 minutes slowly draw the oxygen bubbles into the potometer by pulling the syringe out. Keep drawing water until no more bubbles appear. Make sure the gas bubble is within the measurement tube. If the bubble is too long, then measure the length of half the bubble and pull it out of t he measurement tube to let the other half of the bubble to be measured. (*The syringe has to be pulled slowly because if it is pulled too hard then the bubbles can escape the measurement tube and can enter the syringe and pushing them back will not provide a very accurate result.)*
15. Note down the results i.e. the length of the air bubble. Made sure the result is as accurate as possible by minimising human errors.
16. Repeat with the same elodea 2 times with the same temperature. *(Same elodea should be used as it h as the same amount of leaves and therefore the surface area available for photosynthesis is the same Repeating the experiment twice would increase reliability of the results)*
17. Repeat steps 10-16 for other temperatures. In case of high temperatures like 45 ^{o}C make sure the test tube with the elodea is inserted into the water bath at least halfway as in the beaker. Also check the temperature of the water bath before and during the experiment. This should be done to make sure that the temperature is the one needed before inserting the elodea and also it remains constant throughout. Change the temperature as and when needed.
18. Convert the length calculated to volume using the formula – $\pi r^2 l$ where l is 0.8mm and note down the result. Then divide the volume of oxygen produced by 5 as 5 minutes were allowed for photosynthesis to obtain the rate of oxygen production (mm^3/min). Then calculate the average of the rate of bubbles produced by dividing the sum of the repeats for each temperature by 3.

Key Variables:
The factors to be changed are:

Variable	How will it be changed
Temperature	The aim of the experiment is to observe the changes in the rate of photosynthesis when subjected to change in temperatures. So the temperature has to be changed to observe its affect on the rate of photosynthesis. This would be done by using different water baths set at different temperatures. Ice water will be used to obtain low temperatures if they cannot be achieved in a water bath. A thermometer will be used to ensure the temperature of the water bath stays constant and any changes will be made as necessary. All other factors which might ideally affect the rate of photosynthesis will be kept constant. These are shown below.

The factors that will be kept the same are:

Variable	How will it be kept constant
Light Intensity	The light intensity would be kept constant throughout the experiment using the same lamp for each repeat. All other sources of natural or artificial light will be blocked or switched off so that the lamp being used is the only source providing the light energy to the elodea. Furthermore, the lamp would be kept at the same distance from the test tube containing the elodea so that the intensity of the light stays the same for each temperature. The distance will be measured using a ruler (correct to 1mm). The ruler may account for an inaccurate measurement of the distance so human errors will have to be eliminated while performing this task.
Elodea	The size of the elodea being used is the key factor as it is directly proportional to the rate of photosynthesis. This would be done by using the same size- length of the elodea (50mm) throughout the experiment. For each individual temperature, same piece of elodea would be used. Again the length of the elodea will be measured using a ruler accurate to 1mm.
Amount of time to let photosynthesis to occur	The amount of time given to each elodea, to allow photosynthesis to occur and the air bubble to be produced will be kept the same. This is because if more time is allowed for photosynthesis to take place, a larger air bubble will be produced. If a smaller amount of time is allowed, then a smaller air bubble will be produced. Again the rate of photosynthesis will be affected by the amount of time given for the reaction to occur and not only temperature. The amount of time given would be 5 minutes each.
Amount of time to let the elodea to acclimatise	Firstly all the elodea would be kept underwater and in front of a light source to let it adapt toe the environment and to make conditions idea for survival. Same amount of time would be allowed for each piece of parent elodea from which the different lengths of elodea would be cut. These would be kept overnight for approximately 8 hours. Then, during the actual experiment, when the elodeas are introduced to a new light source, 2 minutes would be allowed to let the plant accustom to the change in light intensity.

Elodea water	The water to be used to immerse the elodea into during the experiment would be the same water that the parent elodeas were left in to acclimatise overnight. The elodea would already be familiar with the water potential and the temperature of the surrounding and would already be in the process of carrying out photosynthesis.
Carbon dioxide concentration	A higher amount of carbon dioxide means a higher rate of photosynthesis and vice versa. To eliminate carbon dioxide concentration from being a limiting factor, same amount of sodium hydrogen bicarbonate will be added to each test tube containing the elodea so that carbon dioxide is present in abundance.

Safety: The following safety measures have to be kept in mind while performing the experiment:

- Goggles should be worn while handling chemicals like Sodium Hydrogen bicarbonate which can be irritant to skin or eyes. Powdered chemicals should be handled using a spatula and the liquid chemicals should be poured carefully using a pipette.
- Elodea water can also be irritant or infectious if came into contact with naked skin or eyes. Goggles again should be worn while pouring elodea water into test tubes which again should be done using a pipette. Tweezers should be used and gloves should be worn while using tweezers to pull the elodea out of the pondweed water to avoid any contact of the water with bare skin.
- The light source i.e. the lamp should be kept at a safe distance from water or any other liquid apparatus to prevent electrocution or malfunctioning of the lamp. The same applies for any other electrical equipment like the digital clock because it can stop working if it comes in contact with a liquid. Additional precautions should be taken while handling the light source because gradually it will get heated and carries a danger of burning skin if handled incorrectly.
- Care should also be taken while handling sharp instruments like the scalpel to prevent any physical injuries.

Results:

Table showing the results obtained from the experiments conducted individually at different temperatures:

Repeat	Temperature (^oC)	Light Intensity (LUX) A-Lowest H-Highest	Length of O_2 released in 5 mins (mm)	Volume of O_2 released in 5 mins (mm^3/5mins)	Rate of O_2 released (mm^3/min)	Average rate of O_2 released (mm^3/min)
1		H	0.00	0.00	0.00	
2	0	H	0.00	0.00	0.00	0.13
3		H	1.00	2.01	0.40	
1		H	5.00	10.05	2.01	
2	15	H	3.00	6.03	1.21	1.61
3		H	4.00	8.04	1.61	
1		H	60.00	120.64	24.13	
2	25	H	70.00	140.74	28.15	27.48
3		H	75.00	150.75	30.15	
1		H	100.00	201.06	40.21	
2	35	H	130.00	261.38	52.28	47.58
3		H	125.00	251.33	50.27	
1		H	160.00	321.70	64.34	
2	45	H	123.00	247.31	49.46	58.71
3		H	155.00	311.65	62.33	
1		H	8.00	16.08	3.22	
2	55	H	6.00	12.06	2.41	3.22
3		H	10.00	20.12	4.02	
					0.00	
1		H	2.00	4.02	0.80	
2	65	H	6.00	12.06	2.41	2.14
3		H	8.00	16.08	3.22	

Class Results:

Table showing the individual average results and the class average rate of oxygen production at each temperature:

Temperature (^oC)	Group												Class Average of Average rate of O_2 released (mm^3/min)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	
0	0.40	0.10	0.07	0.10	0.13	0.08	0.10	0.10	0.10	0.40	0.13	0.27	0.40	0.18
15	10.59	3.72	8.20	4.32	1.61	2.71	3.12	13.04	1.91	5.50	2.01	2.14	8.54	5.19
25	14.07	10.35	20.20	11.16	27.48	8.90	18.80	29.91	2.42	25.47	18.23	23.46	20.65	17.78
35	20.44	32.97	44.70	33.28	47.59	33.88	41.93	43.35	6.53	27.21	35.39	44.64	33.64	34.27
45	38.30	31.16	48.30	34.28	58.71	36.49	30.36	37.33	20.80	34.72	52.54	49.73	57.10	40.76
55	25.07	11.16	8.30	12.87	3.22	9.25	17.19	19.67	18.70	8.85	12.05	22.25	12.73	13.95
65	14.75	1.41	2.70	7.34	2.14	0.90	5.13	7.55	16.40	2.95	1.20	0.94	0.67	4.93

Statistical Analysis:

The t-test assesses whether the means of two groups of data significantly different from each other or not. In this experiment the t-test will be used to asses the level of significance between the average rates of production of oxygen between two different temperatures. The t-test values will provide a statistical value supporting the affect of temperature on the rate of photosynthesis. as the values to be taken are averages, they would be taken from the table showing results of class averages.

The steps to calculate the t-values for two sets of data are:

1. Work out the average (mean) for both sets of data.
2. Subtract the smaller value of the mean from the larger value
3. Work out the standard deviation of one set of data and square the number and divide it by the number of pieces of data i.e. n.

The following formula is used to calculate the standard deviation used in the t test:

$$S_{\chi} = \sqrt{\frac{(\Sigma\chi^2) - \frac{(\Sigma\chi)^2}{n}}{n-1}}$$

Σ = Sum of…
χ = individual value of average rate of oxygen produced
n = total number of average rates of oxygen production

4. Work out the standard deviation of the other set of data using the formula given above. Square this result number and then divide it by n.
5. Add together the figures calculated in step 3 and 4.
6. Take the square root of the answer calculated in step 5.
7. Divide the difference between the two means (Step2) by the figure calculated in step 6. This is the t value.

Now use the table of t-values to make deductions.

The column "Degrees of freedom" on the table represents the total number of data reduced by 2. As there are 13 sets of data, the degree of freedom will be (13x2)-2 which is 25. The level of confidence is 5% with corresponding critical value being 2.06

If the calculated value for t is above the critical value of t i.e. 2.06, at confidence level 5% which shows 5% probability that chance could have produced it, on the table, it can be concluded by more than 95% confidence that the difference between the means of the two temperatures is significant showing that the results are statistically correct.

If the t value is smaller than 2.06 i.e. the corresponding critical value of t (confidence level 5%), it can be deduced that by less than 95% confidence that the difference between the two means is significant i.e. the results are statistically incorrect.

Table of t test values (the values are correct to 2 decimal places):

Degrees of freedom	**Value of t**			
1	6.31	12.7	63.7	636
2	2. 2	.3	. 3	31.6
3	2.3	3.1	.	12.
	2.13	2.7	.6	.61
	2. 2	2. 7	. 3	6. 7
6	1.	2.	3.71	. 6
7	1.	2.37	3.	. 1
	1. 6	2.31	3.36	.
	1. 3	2.26	3.2	.7
1	1. 1	2.23	3.17	.
11	1.	2.2	3.11	.
12	1.7	2.1	3. 6	.32
13	1.77	2.16	3. 1	.22
1	1.76	2.1	2.	.1
1	1.7	2.13	2.	. 7
16	1.7	2.12	2. 2	. 2
17	1.7	2.11	2.	3. 7
1	1.73	2.1	2.	3. 2
1	1.73	2.	2. 6	3.
2	1.73	2.	2.	3.
22	1.72	2. 7	2. 2	3.7
2	1.71	2. 6	2.	3.7
26	1.71	2. 6	2.7	3.71
2	1.7	2.	2.76	3.67
3	1.7	2.	2.7	3.6
	1.6	2. 2	2.7	3.
	1.6	2. 1	2.7	3. 2
6	1.67	2	2.66	3. 6
7	1.67	1.	2.6	3.
	1.66	1.	2.6	3. 2
	1.66	1.	2.63	3.
1	1.66	1.	2.63	3.3
Probability that chance could have produced this value of t	.1	.	. 1	. 1
Confidence level	1 %	%	1%	.1 %

Conclusion:
A clear bell shape curve is observed from both the graphs showing the average rate of oxygen production plotted against temperature. In the graph showing individual results, it can be seen that as the temperature increases from 0 ^{o}C to 35 ^{o}C the rate of oxygen production increases. It reaches a peak at a temperature between 35 ^{o}C -45 ^{o}C and then the rate of oxygen production decreases from 45 ^{o}C to 65 ^{o}C. The lowest values are record at 0 ^{o}C and 65 ^{o}C This is the general trend of the graph and proves that as the temperature is increased, so does the rate of photosynthesis but after a certain temperature (the optimum temperature which forms the peak), the rate of photosynthesis decreases. Although one anomalous result is present on the graph at 25 ^{o}C, rest of the points lie very close or on the line of best fit and this shows that the result are reliable.

The line of best fit shows that as the temperature is raised from 0 ^{o}C to 15 ^{o}C the average rate of oxygen production increases from 0.5 mm^3/min to 47.5 mm^3/min. So it can be concluded that as the temperature increase from 0 ^{o}C to 35 ^{o}C, so does the average rate of oxygen production and therefore so does the rate of photosynthesis. But a closer look at the graph shows a difference in the increases between 0 ^{o}C and 15 ^{o}C, 15 ^{o}C and 35 ^{o}C and 25 ^{o}C and 35 ^{o}C. As seen from the results table, the light intensity of the apparatus provided by the lamp was constant at H for temperatures between 0 ^{o}C to 35 ^{o}C, so light intensity was not a limiting factor. Enough Sodium hydrogen carbonate was also added to provide carbon dioxide to the elodea in excess which prohibited it from being a limiting factor. So only the change in temperature affected the rate of photosynthesis between 0 ^{o}C and 35 ^{o}C.

The lowest value for the rate of oxygen production is at 0 ^{o}C that of 0.5 mm^3/min. As the temperature rises from 0 ^{o}C to 15 ^{o}C, the rate of oxygen production increases from 0.5 mm^3/min to 6 mm^3/min showing an increase of 5.5 mm3/min. Then as the temperature increases from 15 ^{o}C to 25 ^{o}C, the average rate of oxygen production does increase but shows a steeper change i.e. from 5.5 mm^3/min at 15 ^{o}C to 20 mm^3/min at 25 ^{o}C. This is a large increase of 14.5 mm^3/min compared to the increase of 5.5 mm^3/min from 0 ^{o}C to 15 ^{o}C. This proves the prediction wrong which stated that "as the temperature increases from 0 ^{o}C to 35 ^{o}C, the rate of photosynthesis will increase proportionally". It is clear from the graph that the gradient of the change between 0 ^{o}C to 15 ^{o}C is much less steeper than the gradient of the change from 15 ^{o}C to 25 ^{o}C. This proves that temperature has a larger affect on the rate of photosynthesis between 15 ^{o}C to 25 ^{o}C than at temperatures between 0 ^{o}C to 15 ^{o}C. If the change were to be proportional, the changes in the rate of oxygen production would have been constant between 0 ^{o}C to 15 ^{o}C and 15 ^{o}C to 25 ^{o}C i.e. the gradient would have been constant. The change between the rate of oxygen production between 25 ^{o}C and 35 ^{o}C further proves the prediction incorrect because the change between these two temperature is an even higher one that of 24.75 mm^3/min. Therefore it can be concluded that as temperature is increased, the change in oxygen production (therefore the rate of photosynthesis) is not constant, but the quantity of the change in the rate of photosynthesis increases as the temperature increases to 35 ^{o}C.

The personal graph shows an increase in the rate of oxygen production until approximately 42.7 ^{o}C. After this temperature the graph begins to level off. This temperature of 42.7 ^{o}C is the optimum temperature where all the enzymes involved in photosynthesis are working at their maximum capacity. The rate of oxygen production

at this point is 59 mm^3/min . At the optimum temperature, temperature is no longer the limiting factor.

After the peak formed 42.7 ^oC, the graph showing individual results shows a decrease in the rate of oxygen production as the temperature is increased. The rate of oxygen released recorded at 45 ^oC was 58.25 mm^3/min and at 65 ^oC was 1.3 mm^3/min. It is clear from the curve that as the temperature increases from 45 ^oC and 65 ^oC there is a steep decline in the gradient showing the decrease in the average rate of oxygen production. Therefore the prediction which stated that after the optimum temperature, an increase in temperature will result in a decrease in the average rate of photosynthesis between 45 ^oC and 65 ^oC is proven correct. Between 45 ^oC to 65 ^oC, too the light intensity was constant at H and again Sodium hydrogen carbonate was providing carbon dioxide in plenty, eliminating the risk of them affecting the rate of photosynthesis.

But again the rate of decrease between 45 ^oC to 55 ^oC and 55 ^oC to 65 ^oC is not constant and therefore again the part of the prediction that stated that the decrease between 45 ^oC to 65 ^oC would be inversely proportional to the increase in temperature is proved wrong to an extent.

The value of the rate of oxygen produced from the personal graph is 58.35 mm^3/min. As the temperature increased to 55 ^oC the rate of oxygen production dropped to 5.75 mm^3/min, showing a decrease of 52.6 mm^3/min. whereas between 55 ^oC and 65 ^oC, a small decrease of 3.75 mm^3/min was recorded. From the graph it is clear that the curve is very steep between 45 ^oC and 55 ^oC, whereas it levels off considerably between 55 ^oC and 65 ^oC. This graphical evidence and the clear difference in gradients of the changes between the two sets of data shows that an increase in temperature has a greater affect on rate of photosynthesis between 45 ^oC and 55 ^oC compared to the smaller affect it produces between temperatures within 55 ^oC and 65 ^oC.

For the graph showing the average rate of bubbles produced for the whole class, the shape too is of a curved bell. Again the lowest values are at 0 ^oC and 65 ^oC. The optimum temperature is recorded at 43.6 ^oC and the rate of oxygen produced is 41.7 mm^3/min. This graph too like the graph showing individual results starts off at a very low rate (0.3mm/min), and then it gradually increases to 5.2 mm^3/min at 15 ^oC (like the previous graph). After 15 ^oC again, there is a steep increase in the rate of oxygen production, till it reaches 43.6 ^oC which is the optimum temperature recording the highest amount of oxygen released (41.7 mm^3/min). This graph too proves that as the temperature is increased from 0 ^oC to 35 ^oC, so does the rate of photosynthesis, but again between these temperatures, the curve between 25 ^oC and 35 ^oC is the steepest gradient and the curve between 0 ^oC and 15 ^oC showing the shallowest gradient. The general trend after the optimum temperature is similar to the personal graph showing a decrease in rate of oxygen production with an increase in temperature. As the temperature is increased beyond the optimum temperature there is a steep decline in the average rate of oxygen production from 45 ^oC to 55 ^oC and then the decrease becomes less steep until it reaches 65 ^oC. This trend was similar to the graph showing individual results proving the hypothesis, which stated the decrease in rate of photosynthesis after the optimum temperature will be constant, incorrect. Overall this graph too showed reliable results as most of the points lay very close or on the line of

best fit. But an anomaly was present at 25 ^{o}C (same as on the personal graph) as it lay away from the line of best fit.

Therefore, the results obtained were in accordance with the predictions made. The prediction stated that "As the temperature increases from 0 ^{o}C to 35 ^{o}C, the rate of photosynthesis will increase proportionally (directly proportional to increase in temperature". This was proven true up to a certain extent as the general trend depicted by both graphs was that of a proportional increase in the rate of photosynthesis as the temperature rose from 0 ^{o}C to 35 ^{o}C. But it wasn't predicted that the rise in the rate of photosynthesis from 0 ^{o}C to 15 ^{o}C would be less steep than the rise between 15 ^{o}C -25 ^{o}C and 25 ^{o}C -35 ^{o}C. Also as predicted at 0 ^{o}C the elodea showed the lowest rate of oxygen production whereas at 35 ^{o}C it showed the highest within that range for both the graphs.
The optimum temperature was predicted to be 37.5 ^{o}C but the personal graph showed a peak at 42.7 ^{o}C and the graph showing the class averages formed a peak at 43.6 ^{o}C approximately. Although the peak was formed at a much higher temperature, but both the optimum temperatures were similar to each other and did lie within the range 35 ^{o}C -45 ^{o}C as predicted
For the temperatures between 45 ^{o}C and 65 ^{o}C, it was predicted that "the rate of photosynthesis will decrease with it being the highest at 45 ^{o}C and lowest at 65 ^{o}C and the decrease will be inversely proportional to the increase in temperature". This was also proven right as the rate of oxygen production did fall between 45 ^{o}C and 65 ^{o}C for both graphs. Also as per the prediction, the highest value was recorded at 45 ^{o}C and the lowest value was recorded at 65 ^{o}C in the individual and the class average results. But again as the decrease was inversely proportional to the increase in temperature, but there was a noticeable difference between steepness of the curves between 45 ^{o}C and 55 ^{o}C and 55 ^{o}C and 65 ^{o}C in both the graphs.

The t test values showed how significant the differences in the mean averages of oxygen production between two chosen sets of temperatures were. The values obtained were ads follows:

<u>0^{o}C – 35^{o}C</u>
The t test value obtained was 10.81. This value is clearly above critical value of 2.06 showing that it can be said with at least 95% confidence that there is a significant difference between the means of 0^{o}C (0.18mm^{3}/min) and 35^{o}C (34.27mm^{3}/min). The null hypothesis stating that there is no significant difference between the means of the two temperatures is rejected. As these values are statistically correct, it can be concluded that these temperatures did have an effect on the rate of oxygen production and in agreeing with the prediction, therefore caused an increase in rate of photosynthesis.

<u>35^{o}C – 65^{o}C</u>
The t test value was 8.44. this value clearly above the critical value of 2.06 showing that it can be said with at least 95% confidence that there is a significant difference between the means of the two sets of values of 35^{o}C (0.18mm^{3}/min) and 65^{o}C (4.93mm^{3}/min). Therefore the null hypothesis stating that there isn't a significant difference between the means of the two data can be rejected. These values too are statically correct, it can be concluded that temperature has had an effect on the rate of

oxygen production and hence caused the decrease in rate of photosynthesis between these temperatures as predicted and recorded.

<u>25°C – 55°C</u>

The t testy value is 1.36. This value unlike the last two t test values is not greater than the critical value of 2.06 showing that it can be said with less than 95% confidence that there is a significant difference between the means of the two sets of values of 25°C (17.78mm^3/min) and 55°C (13.95mm^3/min). Therefore the null hypothesis stating that there is no significant difference between the two means has to be accepted and unlike the previous two t test values which did disagree with the null hypothesis. It can be concluded that these temperatures did not affect the rate of oxygen production very significantly and therefore had an equal or a similar affect on the rate of photosynthesis.

Discussion:

Between 0 °C and 35 °C, the average rate of oxygen production and therefore the rate of photosynthesis increased as the temperature increased. This shows that the rates at which the reactions involved in photosynthesis were taking place at a higher rate at an increased temperature and therefore forming more products. This increase was caused by the change in temperature (as limiting factors like carbon dioxide concentration and light intensity were controlled). Between these temperatures the light intensity stayed the same (at H) eliminating it from being a limiting factor and sodium hydrogen carbonate was provided in excess so that enough carbon dioxide was available for the reactions.

The reactions involved in the light independent stage solely depend on temperature as their energy source. At a low temperature (0 °C) reactant molecules like the 6 carbon instable intermediate and triose phosphate possess a very low amount of kinetic energy. The reactants move around in the environment very slowly the probability of these molecules colliding isn't very great and if they do collide they do not have sufficient energy to break or form bonds i.e. to overcome activation energy to reach the transition stage and to form any products. Therefore very few or no products are formed at a temperature as low as 0 °C from the light independent reactions. But a higher temperature (15 °C) accounts for a gain in the kinetic energy of the reactant molecules and they start to move with an increased velocity. This increases the probability of them colliding and also provides them with sufficient energy to overcome the activation energy barrier to form the respective products. More products will be formed as more successful collision will be taking place. Therefore at an increased temperature of 15 °C, in the light independent stage, reactions like the carbon fixation and the reduction of glycerate phosphate take place at a higher rate because these reactants gain an increase in their kinetic energy. So they collide more often than at 0 °C, and as they collide with energy higher than the activation energy, they are converted into products like the unstable intermediate and triose phosphate respectively, at a quicker rate. This increases the overall rate of photosynthesis and as the rate of the reactions is higher, more ATP is used up in reactions like regeneration of Ribulose Bisphosphate, and this forms more ADP and Pi. These products are recycled back into the light stage. Although this stage is dependant on light as the energy source, it does require ADP and Pi to form ATP during electron excitation. Therefore as an increased supply of ADP and Pi is available, the light stage uses them to from ATP at an equally higher rate, thereby increasing the rates of reactions like

the photolysis of water (so more oxygen is released which is recorded as an increase in the average rate of oxygen produced in both graphs between 0 ^{o}C to 35 ^{o}C). This has an overall increase in and increase in the products of photosynthesis being formed i.e. oxygen in the light stage and glucose in the dark stage. Therefore an increase in temperature affects the dark stage predominantly but has an indirect affect on increasing the overall rate of photosynthesis.

In both the graphs, the increase in rate of oxygen production recorded between 0 ^{o}C - 15 ^{o}C was less steep than the increase recorded between 15 ^{o}C -25 ^{o}C and 25 ^{o}C -35 ^{o}C. This difference in the gradient was because as the temperature is increase from 0 ^{o}C to 15 ^{o}C, the molecules involved in both the light and the dark stage, are provided with a little amount of kinetic energy which makes them move with a slightly increased velocity. This slightly increased velocity does increase the probability of collisions between reactant molecules to create or break bonds in order to form products, but this gain in kinetic energy is not as significant as the increase experienced by the same reactant molecules at 25 ^{o}C. At 25 ^{o}C a large amount of heat energy is provided by the increased temperature which provides a higher amount of kinetic energy (compared to the energy provided by 15 ^{o}C), which causes more vigorous vibrations within the reactant molecules and the increased velocity improves the chances of them colliding together. Moreover the collision take place with an even higher energy (again compared to the energy they collide with at 15 ^{o}C) and therefore the activation energy is overcome quicker to form the transition stage and thereafter the products too are formed at a much faster rate. This increases the rate of photosynthesis and this increase is even more rapid between 25 ^{o}C and 35 ^{o}C as even more kinetic energy is supplied to the reactants at 35 ^{o}C.

An indirect but a very significant impact of temperature to bring about an increase in the rate of photosynthetic reactions is caused by its influence on enzyme activity. Enzyme activity is only increased between 0 ^{o}C and the optimum temperature of the enzyme (as shown by the general increasing trend shown by the graphs). Enzymes depend on substrate (reactant) molecules binding to their active sites so that a reaction can take place for and enzyme substrate complex can be formed and released. Temporary bonds are formed between the substrate molecules and the enzyme's amino acids. The enzyme changes conditions of its active site and holds the substrate molecules in such a place which makes it easier to break or form bonds as required. This lowers the activation energy required for the enzyme substrate complex to be formed and it is formed easily and at a quicker rate than it normally will. The rate of this induced fit mechanism of the enzymes can be increased by increasing the speed of the random movement of the enzyme and the substrate molecules within a given environment which will increase the probability of the substrate molecules colliding and fitting into the active site of enzymes and forming an enzyme substrate complex. This is done by an increase in the kinetic energy of the molecules which is provided by an increase in temperature. Therefore as the elodea is moved from a relatively cold environment (0 ^{o}C or 15 ^{o}C) into warmer conditions (25 ^{o}C or 35 ^{o}C), the enzymes present in the elodea and the substrate molecules experience a gain in kinetic energy and vibrate more rapidly than at lower temperatures. The probability of the substrate molecules combining with the active site and therefore a reaction to occur is increased. Therefore more products are formed at higher temperatures. In the dark stage, the enzyme Rubisco facilitates fixation of carbon with Ribulose Bisphosphate to form the unstable intermediate (which is further broken down and then those

products are reduced to form glucose). Therefore the substrate for Rubisco is carbon dioxide and Ribulose Bisphosphate molecules. As the temperature is increased from 0 ^{o}C to 25 ^{o}C, the enzyme and both types of substrate molecules gain kinetic energy and start moving faster in the stroma of the chloroplast (the site of dark stage). The possibility of the carbon dioxide and/or the Ribulose Bisphosphate molecules colliding and combing to the active site of the Rubisco molecules is increased due to the increase in velocity. This increases the prospects of the enzyme substrate complex i.e. the unstable intermediate being formed. Therefore the rate at which the reactant (which is now the unstable intermediate) is produced increases, and it also gains more kinetic energy because of the high temperature. This increases the number of successful collisions and the rate at which it is converted to 2 molecules of glycerate phosphate is increased. The glycerate phosphate acts as the reactant and is then reduced to form triose phosphate with the help of ATP and reduced NADP (releasing NADP,ATP and Pi) and this reaction too is quickened by the increase in temperature and by the high rate of glycerate phosphate production. The triose phosphate is then converted to amino acids, lipids and glucose while some of it is regenerated to form Ribulose Bisphosphate with the help of ATP (which is converted to ADP). Therefore the overall rate of photosynthesis is increased.

This increase in the rate of the reaction occurring in the dark stage has an indirect affect in increasing the rate of reactions in the light stage too. As more glycerate phosphate was reduced and more triose phosphate was regenerated, more ATP and reduced NADP were used and more NADP, ATP and Pi were produced to be used in the light stage. Therefore, these products were used at a higher rate in the cyclic and non cyclic photophosphorylation stages in the grana of the chloroplast (site for light dependant reactions) and the rate at which photolysis of water occurred was increased, thereby producing more oxygen i.e. the product. Also a higher temperature (like 25 ^{o}C -35 ^{o}C) has a direct affect on the rate at which the enzyme controlled photolysis reaction occurs within the light stage itself. This is brought about by the increase in kinetic energy of the enzyme and substrate i.e. water molecules, which increase the possibility of them colliding and combing in the active site of the enzyme and eventually the amount and the rate at which the hydrogen ions and water molecules (the products) are released is increased.
As before, at lower temperatures the probability of collisions between enzymes and substrate molecules is low, therefore a low increase in the rate of photosynthesis was observed between 0 ^{o}C and 15 ^{o}C, whereas at higher temperatures, more kinetic energy is provided and the number of successful collisions ids greater, resulting in a larger increase in the rate of photosynthesis between higher temperatures like 15 ^{o}C and 25 ^{o}C and an even higher increase between 25 ^{o}C and 35 ^{o}C.

The optimum temperature is the temperature at which the enzymes are working at their highest capacity. This temperature was recorded at 42.7 ^{o}C in the personal graph and at 43.6 ^{o}C in the class average graph. This is high temperature and therefore the rate of the enzyme controller reactions (and all other reactions) will be very high because of the increased number and strength of collisions between enzyme and substrate molecules (and between the other enzyme independent reactant molecules). All the active sites of the enzymes (in both the dark and the light stage) are full and the maximum amount of product is being released at the highest possible rate. Therefore from the dark stage the maximum amount of carbon dioxide is being fixed by Rubisco and the maximum amount of the unstable intermediate and eventually

glucose is being formed. In the light stage the maximum amount of oxygen is being released by the enzyme controlling the photolysis of water and this high release of oxygen is recorded as the highest values at these temperatures on both the graphs.

As the temperature increased beyond the optimum temperature, both graphs showed a decrease in the average rate of oxygen produced. This reverse affect was caused by the denaturisation of enzymes. Enzymes are globular proteins and have a specific 3 dimensional tertiary structure with a precise active site. At very high temperatures (temperatures higher than their optimum temperature) they gain a very high amount kinetic energy that causes the enzyme molecule to vibrate vigorously. These vibrations cause the bonds like hydrogen bonds, disulphide bonds and the hydrophobic interaction, holding the tertiary structure of the enzyme together, to weaken or to break. The specific structure of the enzyme and especially its active site (which is complementary to the substrate molecule) is distorted. The enzyme is no longer specific to its substrate and no substrate molecules can fit into the active site to form the enzyme substrate complex and therefore the rate of the reaction is decreased. During photosynthesis, the enzyme controlled reactions like photolysis of water and carbon fixation by the enzyme Rubisco, form a low amount of no products at all as they are not catalysed by their respective enzymes anymore. Therefore there is an overall decrease in the amount of unstable intermediate (and then glucose) formed at the dark stage and the amount of oxygen produced in the light stage and this undoubtedly decreased the rate of photosynthesis. The optimum temperature for enzymes like Rubisco is 37.5 ^{o}C and any temperature higher than this will show a decrease in the rate of photosynthesis. Therefore the decrease in the rate of reactions between 45 ^{o}C, 55 ^{o}C and 65 ^{o}C were because of the enzymes being denatured at these extremely high temperatures.

The difference in the steepness of the difference between the rates of oxygen production between 45 ^{o}C to 55 ^{o}C and between 55 ^{o}C to 65 ^{o}C is also related to the enzymes becoming denatured and inactive at the high temperatures. The difference between 45 ^{o}C and 55 ^{o}C degrees was steeper because as the enzymes started to denature (at 45 ^{o}C) the rate of photosynthesis dropped rapidly as it was at its highest point at its optimum temperature. A more rapid decrease in the rate of photosynthesis was observed which is shown by a very steep gradient by the curve between these two points. As the temperature increased from 45 ^{o}C to 55 ^{o}C, more and more enzyme controlled reactions start to slow down and from less enzyme substrate complexes (especially oxygen which was being measured). After 55 ^{o}C, it can be said that most of the enzymes were denatured and most of the reactions catalysed by enzymes like photolysis and carbon fixation ceased to occur completely. Very few or no glucose or oxygen is produced so less oxygen was released form the plant as the temperature increased from 55 ^{o}C to 65 ^{o}C and a shallower gradient was recorded between 55 ^{o}C and 65 ^{o}C of both the graphs.

High temperatures not only denature enzymes but other molecules as well. For instance molecules from the dark stage like glycerate phosphate or triose phosphate could have gained such a high amount of kinetic energy that the excessive vibrations caused an irreversible change in the structures of their molecules preventing them from reacting and forming products like the glucose and amino acids from the triose phosphate. This could also have resulted in less regeneration of the Ribulose Bisphosphate from the triose phosphate and inevitably less carbon fixation would

have taken place and the whole Calvin cycle could have stopped and no more glucose (a product of photosynthesis) would have been formed. If this was the case, then no ADP, Pi and NADP would have been made available to the light stage which in turn would have stopped the cyclic and non cyclic photophosphorylation reactions and eventually no photolysis would have taken place and very little or no oxygen at all would have been released. So no oxygen bubble would have been recorded at these high temperatures and therefore the rate of photosynthesis will have decreased.

Although the enzymes were definitely denatured by the excessive increase in temperatures, there is a possibility that the presence of some external factors resulted in the decrease in enzyme activity. These factors are called the inhibitors which bind to the active site of the enzyme reversibly or irreversibly and slow down or completely denature the enzyme respectively. Inhibitors could have been present as a result of the apparatus not being cleaned completely before the experiments were conducted. As a result traces of molecules from previous experiments could have been left which possibly could have acted as an inhibitor. Maybe some inhibitors could have been present in the elodea water that was used as this water was not purified.

Evaluation:

Anomalous Results*:* Two anomalous results were present, one in each graph and both anomalies were at 25 ^{o}C. Both the anomalies were present above the line of best fit showing a higher amount of oxygen being collected than expected. The reasons for these anomalies could have been:

- *Change of rooms:* As we were unable to perform the experiment in the same room, the environment to which the elodeas used were exposed too, was changed halfway through the experiment. This could have accounted for the elodea giving anomalous values as it had to acclimatise to the new environment.
- *Elodea*: Each elodea piece used was 50mm long and although in the plan it was stated that elodea for each temperature at least, would be cut from the same plant of pondweed. This could not be done for each temperature because sometimes a particular piece of elodea did not photosynthesise or were cut inaccurately and needed to be changed. At higher temperatures of 55 ^{o}C and 65 ^{o}C especially most elodea were denatured after the first or the second repeat and a new elodea had to be used. This elodea was not always from the same parent plant and a different elodea plant could have different number of leaves on it affecting the amount of oxygen released. It could also have been in bad health producing less number of oxygen bubbles at the same given temperature leading to inaccurate results.
- *Errors in measuring the length of the bubble* : Human errors in measuring the length of the bubble produced could have results in the anomalous results. A length higher than the actual length could have been recorded it would showed that the rate of photosynthesis was high even if it was low. This could have been caused when the length of the bubble was larger than the actual measurement tube and had to be calculated separately by dividing it into two parts. Also pulling the syringe required some practice and a lot of caution. Errors could have been caused while pulling the syringe and maybe the bubble may have been sucked into the syringe. Also not all of the bubbles came out as

one single line of water. So any some small bubbles may have been neglected which considerably affected average class results especially.

- *Errors in setting up the apparatus* : Not making the cut end of the elodea and the rubber tubing connecting it to the potometer completely air tight could have allowed air bubbles to pass through and to be measured as a part of the length of oxygen bubble released. This would have accounted for the higher rate of photosynthesis than needed. The cut end of the elodea may have come above the water meniscus in the test tube which again could have resulted in the formation of air bubbles inside the tube. Also the syringe may not have been completely emptied while squeezing it in at the start of the experiment while leaving some air inside it. This could have added on to the length being measured and provided inaccurate results. Also as the elodea was cut under water, it may not have been cut at an angle large enough not to allow air bubbles to be formed. During the experiment this could have results in the accumulation of air bubbles at the end of the shoot of the elodea preventing the oxygen to be released out of the plant and to be measured
- *Acclimatisation time*: The elodea plants were left to acclimatise on water overnight under the action of a lamp for a considerable amount of time (8 hours). Then the elodea were cut and placed in to an environment with a different temperature than the water temperature that they were acclimatised to and in front of a new light source (which was constant). Although a 2 minute acclimatisation time was given to the elodea, it could have been possible especially at very low temperatures that the internal temperature of the elodea was not the same as the surroundings and it could have still been performing photosynthesis at a higher temperature, thereby producing more oxygen than it ids expected to. This would also make the repeats inaccurate because gradually through the experiment, the elodea will acclimatise to the surrounding water temperature and would start producing oxygen at a different rate than its previous repeats even though the environmental temperature is still the same.

Accuracy: Form the personal graph it is clear that most points lay very close to the line of best fit apart from the anomaly at 25 ^{o}C which lies above the line of best fit. Therefore this graph shows the accuracy of the results.
The graph showing average class results is also showed a high level of accuracy as all the points again lie in close correlation to the line of best again with the exception of the result at 25 ^{o}C which lies above the line of best fit.
Comparing the accuracy of the two graphs, it can be said that both of them are similar in accuracy because both the anomalous results are only present at 25 ^{o}C and both the anomalies lie above the line of best fit. Also both graph show the same general trend from 0 ^{o}C to 65 ^{o}C.

(table on next page)

Apparatus	Accuracy
Lamp	The same lamp was used throughout the experiment. This made sure that the light produced was of the same intensity and did not change with change in the light source. This also minimised the risk of light being the limiting factor as it was in plenty and was kept constant. But any manual or electrical fault could have resulted in the light intensity from decreasing or increasing. But this did not happen in the experiment because the light intensity meter was used to measure the light intensity throughout the experiment and it stayed the same value for each investigation. Therefore even if light intensity affects the rate of photosynthesis it would have been of a negligible amount.
Syringe	Only one syringe was used for each investigation. This wasn't very accurate because traces of water from the last experiment or any air bubbles could have been left in the syringe. Although efforts were made to clean the syringe after every experiment to make it fresh for the next one, the risk of any trace of water even used to clean the syringe was always there.
Scalpel	Using different scalpels would not have affected the experiment to a noticeable extent, but to minimise any risk, only one scalpel was used to cut all the potatoes. The experiment would have been made accurate by how accurately the scalpel was used. The scalpel had to be used under water to cut the elodea and it was hard to look at what angle the elodea was being cut. So it can be said that the elodeas used were cut at different angles if measured precisely. Although this would not have affected the rate of photosynthesis significantly.
Ruler	The ruler was accurate to 1mm. Therefore the lengths of the elodea too were accurate to 1mm. Same ruler was used throughout the experiment and therefore any risks of human errors because of change in ruler were minimised. But human errors in measuring the correct length could have been caused although they were kept to a minimum.
Elodea	The same lengths of elodea were used for each repeat for every temperature (i.e. 50mm). This length was kept the same in order to keep the surface area of the elodea similar during each experiment. Although this was not very accurate because any length of the elodea could have different number of leaves even if they were from the same plant, but counting the number of leaves would have been even more inaccurate and more time consuming.
Potometer	The potometer used had a radius of 0.8mm which provided very precise results and the measuring rube attached to it allowed accurate measurements to be taken. But this method of measuring the length of the oxygen bubble was not very accurate especially near the optimum temperature (between 40 ^{o}C -45 ^{o}C) because the length of the bubble released at these temperatures was very large and could not fit in the measuring

	tube in one go, so had to be split into halves and then measured. The expertise in using the syringe was also tested while measuring long bubbles and depended mainly on how accurately the syringe was used and how accurately the results were noted down and combined.
Measuring tube (attached to the potometer)	As mentioned this tube was accurate to 70mm. This length was long enough to measure the length of the oxygen bubble at low temperatures, but at higher temperatures where the length of the bubble was longer than 70mm, this apparatus was not very accurate. It depended on how expertly the syringe can be manoeuvred to make sure only a part of the long bubble stays inside the tube whilst it is being measured, and also to ensure that none of the unmeasured bubble goes back into the test tube containing the elodea.
Test Tubes	All the test tubes provided were the same volume and were normal test tubes (not boiling test tubes). This minimised the risk of any natural factors affecting solutions differently. For e.g. a boiling test tube has a thicker wall and therefore factors like heat would have less affect on the solution inside it. Similarly a solution in a normal test tube with thin walls would be more prone to be affected by heat than a solution in a boiling test tube. Therefore all normal test tubes of equal volumes were used to maintain accuracy of the experiment. All the test tubes were wiped clean before the experiment so that any traces of solutions from previous experiments were erased and they did not affect the experiment being conducted in any way.
Light intensity meter	The light intensity meter only provided two measurements- A for the weakest light intensity and H being the highest light intensity. This was pretty inaccurate because any slight variations in the light intensity could not be detected and any change in the wavelength of light could have resulted in the reactions at the light stage taking place at a higher or slower rate than expected. This could have lead to inaccurate results.
Test tube rack	4 different test tube racks were used to place the test tube with the elodea in it, inside the water baths. Use of test tube racks doesn't affect the rate of photosynthesis in any way. It just provides a surface to hold the solutions and the elodea in a position to let photosynthesis to occur. But the material that the test tube rack was made of could have affected the rate of photosynthesis as it was a conductor. To ensure fair test, the same test tube rack or test tube racks of the same material should have been used.
Digital clock	The digital clock was accurate to 0.01 seconds. This was a very reliable result as the degree of accuracy was high.
Water bath	The water baths were used to create temperatures of 35, 45, 55 and 65. This temperature was kept constant throughout the experiment in the water bath providing pretty accurate results. But in the case of the temperature changing, adjustments had to

	be made the water in the water bath by adding hot or cold water as needed. This was not very accurate because it easily resulted in a temperature higher or lower than the one needed being reached.

Reliability: The results of the individual experiment conducted were pretty reliable to a certain extent. As evident from the graph, the rate of oxygen released was in a strong correlation to the line of best fit not only for the personal results but also for the class averages. Similar trends shown by both graphs (with anomalies at the same temperature) show that any major drawbacks in the experiment affected the whole class and not only the individual results. The repeats were also in good correlation with each other and were similar to the mean rate of bubbles produced per minute. For example, for the results obtained 0 ^{o}C were as follows:

Repeat	Temperature (^{o}C)	Rate of O_2 bubbles (mm^3/min)	Average rate of O_2 bubbles (mm^3/min)
1		0.00	
2	0	0.00	0.13
3		0.40	

Two of these results were exactly the same and the third one showed a small increase of only 0.4 mm^3/min. This shows a reliable result as the three values are identical to each other and the reliability is further proved by the average result value being very close to each of the repeats.

Another example of a set of reliable results is obtained at 55 ^{o}C. The results obtained are 3.22 mm^3/min, 2.41 mm^3/min and 4.02 mm^3/min respectively. The maximum difference was that of 1.61 mm^3/min only (4.02-2.41=1.61). This showed that the results lie within a very close range and have a high level of reliability. The reliability is further proven by the mean of the data i.e. 3.22 mm^3/min. This value is the same as one of the repeat values and the maximum variance from the results is that of 0.81 mm^3/min only showing that the values are consistent.

But some values like the results obtained at 35 ^{o}C are not very reliable owing to the large amount of difference between the individual values and of the values from the mean.

Repeat	Temperature (^{o}C)	Rate of O_2 bubbles (mm^3/min)	Average rate of O_2 bubbles (mm^3/min)
1		40.21	
2	35	52.28	47.58
3		50.27	

The values are significantly different from each other with the maximum variance being 12.07 mm^3/min (52.28 – 40.21=12.07). The average result also shows a difference of 4.7 mm^3/min from the maximum value. Therefore this result showing such a significant deference cannot be said to be completely reliable.

The average of the individual results can be compared to the class averages to further investigate the reliability of all the results.

Temperature (^oC)	Average rate of O_2 bubbles (mm^3/min) (Individual)	Average rate of O_2 bubbles (mm^3/min) (Class)
0	0.13	0.18
15	1.61	5.19
25	27.48	17.78
35	47.58	34.27
45	58.71	40.76
55	3.22	13.95
65	2.14	4.93

Both the individual and class averages show a similar trend. The average rate of oxygen production for individual and class results increases from 0 ^oC to 45 ^oC and from 45 ^oC to 65 ^oC. Some of the values like at 0 ^oC are very similar to each other whereas other values at 15 ^oC and 65 ^oC are different but do not show a large variation. But values at 45 ^oC and 55 ^oC especially show a very large difference with 55 ^oC showing the largest that of 10.73 mm^3/min. But it can be said that temperatures between 25 ^oC to 45 ^oC show a proportional change i.e. the change between the two values at each temperature is ranges between 10 mm^3/min -18 mm^3/min. Therefore the results change in proportion to each other and although it cannot be said that the results are completely reliable, they do show a fairly good level of reliability when compared. The result at 55 ^oC can be deemed anomalous.

Limitations: The limitations present in the experiment were:

- *Pondweed availability*: This was a limitation because small lengths of elodea plants were provided which were not sufficient to be used in case of a mishap. For example if a pondweed was denatured at high temperatures, not enough pondweed was available to restart the whole experiment for that particular temperature again. So a different elodea length from a different parent plant had to be used. This plant could have had less number of leaves for the same length (50mm) or it was not a healthy plant. This reduced the reliability and the accuracy of the experiment considerably.
- *Collection of oxygen*: The end of the potometer was attached to end of the stem of the elodea. As we know most of the oxygen is released from the stomata pores in the lower epidermis of the leaves. Therefore not all of the oxygen produced by the plant during photosynthesis was measured. Also one cannot be sure whether the oxygen being released from the stem is actually the oxygen produced in photosynthesis by the photolysis of water which takes place in the leaves and not in the stem. But it was not possible to collect the amount of oxygen produced by the leaves with the apparatus provided to us and therefore it was assumed that all of the oxygen being released was a product of photosynthesis.
- *Length of elodea*: The elodea was but underwater and this made it difficult to cut it to a high level of precision and to cut it at an angle sufficient enough to prevent any air bubbles to form. Therefore the elodea could not be cut to an exact length of 50mm and this influenced the reliability and the accuracy of the results in the experiment.

- *Length of the measuring tube*: The length of the measuring tube provided was only 70mm. therefore any air bubble larger than this length had to be split into two parts and then measured. This was not very accurate because photosynthesis was still occurring in the plant and it could not be determined whether more oxygen was being added to the length of the air bubble in contact with the end of the elodea. This way of measuring also depended on how precisely the syringe is handled and to make sure accurate lengths of elodea are sucked into the syringe especially when measuring a very large length of air bubble.
- *Preparing low temperatures*: Method to prepare apparatus at 0 ^{o}C and 15 ^{o}C was very inaccurate because firstly ice had to be used to obtain such a low temperature, but then ice had to be added constantly throughout to maintain the temperature at 0 ^{o}C and 15 ^{o}C because the ice kept melting down. As a result some results could have been exposed to a higher or lower temperature than 0 ^{o}C or 15 ^{o}C. Although the temperature variance would not have been very large, but the reliability of the experiment was reduced. The water baths maintained the high temperature throughout, but if for any reason the temperature was altered, hot or cold water had to be added as per required. Human errors could have caused an increase or decreased temperature being formed as the alterations depended completely on human judgement.
- *Number of repeats*: Seven different temperatures with three repeats were enough to obtain reliable results, but during the time given, only three repeats were possible. This can be said to be a limitation as a higher number of repeats would have provided far reliable results and the changes could have been studied in greater depth.

Significant limitation: The most significant limitation was the elodea itself:
It was planned that the same elodea length would be used for all the repeats at a given temperature. This proved unreliable because firstly using different elodea from different parent plants for each different temperature because the changes in the different elodea could not be pointed out even though all of them were left to acclimatise under similar conditions for the same length of time. Factors like different amount of leaves per length or the health of the plant could have affected the number amount of oxygen produced.

Also the oxygen released from the shoot was being measured and not the amount released from the site of photosynthesis i.e. the leaves. This was inaccurate as firstly the shoot may have been cut at an angle which allowed air bubbles to clog up the cut end. This would have caused any produced oxygen not to be let out of the plant. Therefore not all the oxygen being produced as a result of photolysis of water in the light stage was being measured.

The elodea is a respiring plant and it uses the oxygen produced form photosynthesis for respiration. This could have resulted in some of the oxygen not been released as it was being used up. Therefore an assumption was made that all the oxygen being produced is being released, but this wasn't the case and theoretically it would have lead to inaccuracy in measurements.

Errors: Many errors were observed during the experiment:

- Human error could have been caused during cutting the elodea length under water. An increase or decrease in the length would have results in a higher or a lower amount of oxygen being released respectively because of the changes in the number of leaves in the plant. The elodea plant may not have been cut at an angle sufficient enough to prevent any air bubbles from forming. Any accumulation of air bubbles could have results in sum of the oxygen staying trapped within the plant itself and not being recorded.
- Inaccurate reading of the length of the air bubble released into the potometer could have been caused again by human errors. A larger or a smaller length could have been recorded leading to inaccurate results.
- Human error in handling the syringe could have results in pulling an air bubble too hard so that it is sucked into the syringe without being measured. High degree of expertise was required in measuring lengths of air bubbles larger than the measurement tube attached to the potometer. Any incompetence could have resulted in an inaccurate length being measured affecting the accuracy and reliability of the experiment.
- As mentioned before any fault in the apparatus especially the electrical equipment provided could have caused an inaccurate measurement of results. For instance the lamp could have been faulty and could have been providing varying light intensities at different temperatures, thereby affecting the light stage considerably. The heat provided by the lamp could also have affected the overall temperature of the apparatus especially during low temperatures where it could have caused the ice to melt and provide a need to maintain the temperature using human judgement which cannot be 100% accurate.

Improvements: Certain improvements can be made to improve the reliability of this experiment:

- *Larger length of elodea*: A large enough length of elodea should be provided sufficient to be used for each of the repeats for each individual temperature more than once at least. This should be true especially for higher temperatures where the elodea plant is easily denatured and a new plant will almost certainly be required. If the second elodea length is from a different parent plant, questions about the health and the number of leaves per given length of the plant can be raised.
- If the same experiment were to be repeated the method of preparing the elodea for investigation can be improved. The elodea can be placed in a large trough of water rather than in a beaker. This would make it easier o cut the elodea to the accurate length needed and also to obtain a more accurate angle at the end of the shoot.
- Improvements to the potometer can be made. For instance a larger measuring tube can be provided which would easily accommodate a relatively large length of oxygen bubble. This would not only improve the degree of measurements, but the syringe too can be used with more precision because the chances of small air bubbles being sucked into the syringe will be decreased. If possible a measuring tube accurate to 1decimal place can be provided for more accurate results.
- A digital light intensity meter can be use instead of an analogue one. A digital light intensity meter would provide us with information on a slight change in

the light intensity. Even if the light intensity cannot be kept constant during the experiment, having accurate information on how it changed would lead to better and more accurate conclusions from the results.

- To record any changes in the temperature of the apparatus, instead of using a thermometer, a data logger attached to a temperature sensor can be used. It will record any changes in the temperature throughout the experiment. These changes will be more accurate and far more accurate changes to the temperature can be made as required.
- For more accuracy, a digital clock with a higher degree of accuracy (say correct to 0.001 seconds) can be used which would provide more accurate timing throughout the experiment.
- For more accuracy the same elodea plant should be used throughout the experiment as the number of leaves or the surface area of the plant will not be a limiting factor. To achieve this, firstly human errors will have to be eliminated in preparing the elodea plant i.e. cutting the shoot at an angle and measuring the length exactly. Then the experiment should be carried out in increasing order of temperatures i.e. starting from 0 ^{o}C and finishing at 65 ^{o}C. To prevent the risk of enzymes in the elodea being inactive and denatured after a high temperature like 55 ^{o}C which would cause the same elodea to be unavailable to be used for 65 ^{o}C, less number of temperatures with a higher number of repeats can be used. These temperatures can be much closer together, for instance at a difference of 5 ^{o}C each instead of 15 ^{o}C. So the experiment temperatures can be 10 ^{o}C, 20 ^{o}C, 30 ^{o}C, 40 ^{o}C, 50 ^{o}C with 4 repeats each.

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