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Experiment to Investigate the Effect of Temperature on the Rate of Photosynthesis in *Elodea*

Aim

The aim of this experiment is to investigate the effect of temperature on the rate of photosynthesis in *Elodea* (Canadian pondweed).

Introduction

Elodea is native to North America and is naturalized in Australia, Asia and Europe. It grows completely submerged beneath the water. It has multi-branched, slightly brittle stems that are clothed with whorls of sessile, medium green, pointed leaves.

Elodea grows better in cooler water. In temperatures above 70º F, it becomes spindly. This plant can be grown under 8 to 10 feet of water and will easily reach the surface of the average water garden. This plant derives most of its nourishment from the water through its leaves; its roots serve mainly to anchor it to the bottom, therefore, they may be planted in sand or pea gravel as well as soil. They may be anchored down and just dropped into the pond or planted in containers of sand or gravel. If they are only floated in the pond, too much sunlight and air will kill it.

Photosynthesis is a process which occurs in all green plants, synthesising glucose as a form of energy to be used by the plant. Glucose (and oxygen – a waste product) are produced from a reaction between carbon dioxide and water. The general equation for photosynthesis is shown below.

$$6CO_2 + 6H_2O \longrightarrow 6O_2 + C_6H_{12}O_2$$

To get from the reactants to the products, there are complex processes. The processes are the light dependant stage and the light independent (Calvin cycle) stage. These two stages are on a cycle and rely on each other. Both processes are essential for photosynthesis.

Variables

The rate of photosynthesis can be measured as the volume of carbon dioxide taken in by a plant per unit time. In laboratory investigations the rate is often estimated as the volume of oxygen released per unit time, which is more easily measured. However this is not an accurate method of measuring the rate of photosynthesis. This is because some of the oxygen produced by the plant in photosynthesis is then used in the plants respiration. Respiration is ongoing within plant cells, therefore oxygen liberation from a plant does not measure all the oxygen produced during photosynthesis. The rate of photosynthesis is measured above a point called the compensation point. This is *the point at which the rate of photosynthesis in a plant is in exact balance with the rate of respiration, so there is no net exchange of carbon dioxide or oxygen.'*

The rate of photosynthesis is limited by the factor in the shortest supply. The law of limiting factors states that *when a physiological process depends on more than one essential factor being favourable, its rate at any given moment is limited by the factor at its least favourable value and by that factor alone.* Increasing the factor in shortest supply will increase the rate of photosynthesis, until another factor becomes limiting. If we supply the plant with all of the optimum conditions it needs, the rate of photosynthesis will be limited by factors within the plant, such as the number and location of chloroplasts. Photosynthesis is affected by many factors, both internal and external.

External factors include:

- **Light intensity** – The rate of photosynthesis is directly proportional to the light intensity. The graph below illustrates the relationship between light intensity and rate of photosynthesis.

(Cambridge Biology 1)

The graph levels off because the photosynthetic pigments have become saturated with light, and there is a limiting factor to prevent the reaction from going any faster.

The light intensity at which the rate of photosynthesis is balanced by the rate of respiration is called the light compensation point. This point varies for different plants, depending on whether the plant is classified as a sun plant or a shade plant.

Light intensity only affects the light dependant stage of photosynthesis, but this in turn affects the light independent stage. Light is the driving factor in photosynthesis, as with no light photosynthesis would not take place. The more light there is, the more light is absorbed by the two photosystems. The means a greater amount of electron excitation and consequently more ATP and NADPH is synthesised in the light dependant reaction. Due to the increased amounts of ATP and NADPH, GP is converted to GALP more frequently and the rate of this process is increased, in turn increasing the synthesis of glucose.

The light intensity will be controlled in this experiment by keeping the lamp the same distance from the *Elodea* at all temperatures, ensuring the light intensity is constant throughout the experiment. This actual distance will be determined after the preliminary investigation.

- **Carbon dioxide levels –** the concentration of carbon dioxide in the air is 0.04%. An increase in carbon dioxide concentration up to 0.5% usually results in an increase in the rate of photosynthesis. However the leaves of the plant can be damaged in air containing more than 0.1% carbon dioxide, so the optimum rate of

photosynthesis occurs when the concentration of carbon dioxide in the air is just below 0.1%. The graph below illustrates the effect if carbon dioxide levels on the rate of photosynthesis. Carbon dioxide is involved in fixing wit RuBP, therefore the greater the concentration of carbon dioxide in the air the greater the rate of fixation. The demand for ATP and NADPH will be higher because the rate oh production of GP is higher. Because of this the light dependant reaction will take place at a greater to rate to cope with the demand. The concentration of carbon dioxide will be kept constant by keeping constant the concentration of the hydrogen carbonate solution constant throughout the experiment. The actual concentration to be used in the experiment will be determined after the preliminary investigation. Below is a graph showing the effect of carbon dioxide concentration on the rate of photosynthesis:

(Cambridge Biology 1)

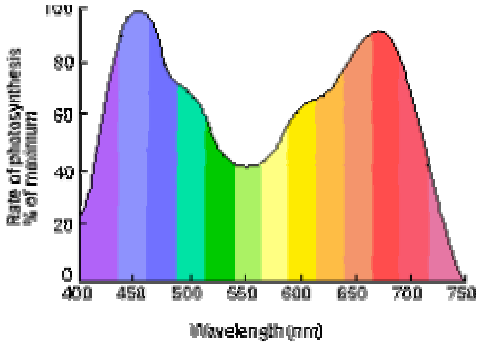
- **Temperature –** The light-dependant stage of photosynthesis is hardly affected by changes in temperature because this stage is driven by light as opposed to temperature. However temperature affects the Calvin cycle (light independent). Reactions in the Calvin cycle are catalysed by enzymes, which are affected by temperature. If the light independent reaction slows down, the demand for ATP and NADPH decreases, slowing down the light dependant stage. Temperature has a big effect on enzyme-controlled reactions. As with all enzymes, there is an optimum temperature at which they work. As plants are not usually exposed to very high temperatures, enzymes which work at an optimum of around 40^0C, such as those in mammals, would be inefficient. The optimum temperature for enzymes within plants is around 30^0C. Up to this temperature, the rate of reaction increases, and then starts to decrease fairly rapidly from this temperature to the point of the enzyme being denatured. For every 10^0C rise in temperature up to the optimum, the rate of reaction approximately doubles. This is known as the Q10 value. The reason behind this is that for every 10^0C rise in temperature, the molecules have twice as much kinetic energy. This causes the molecules to move around twice as fast, increasing the chances of a collision between enzyme and substrate molecules by two. Also, when they do collide, the chance of fixation is double because the force of the collision is twice as great. In this case, the substrate molecules (RuBP and carbon dioxide) and the enzyme molecules (rubsico) gain more kinetic energy with the increase in temperature. As the temperature goes beyond the optimum temperature of the enzyme, the rate of reaction decreases. This is because the kinetic energy is too great and some of the bonds within the enzyme start to break. As the temperature is further increased more of the internal bonds are broken. This causes the shape of the active site to deform so the substrate molecules can no longer fit it. This is the variable that is

going to be investigated in the experiment. The way in which the temperature will be varied will be discussed in the method later on in the investigation.

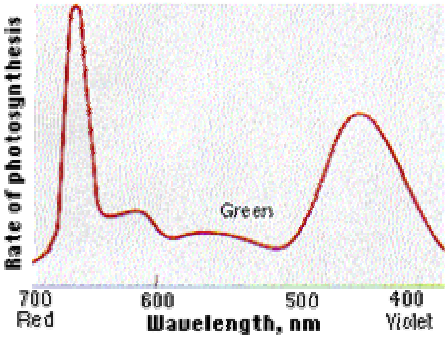
- **Water availability** – Water is essential in photosynthesis to keep the stomata open. If there is not enough water, the stomata will close up, depriving the plant of the carbon dioxide it needs for photosynthesis. Water is involved in photolysis, therefore the more water there is, the greater the amount of hydrogen ions produced from the photolysis of water. Hydrogen ions reduce NADP to NADPH, so the greater the amount of water, the more NADPH is produced. If the amount of water available doubles, twice as much NADPH and ATP is produced, therefore the reaction takes place at double the speed.

- **Light wavelength** – An action spectrum is the rate of a physiological activity plotted against wavelength of light. In 1881, the German plant physiologist T. W. Engelmann placed a filamentous green alga under the microscope and illuminated it with a tiny spectrum of visible light. In the medium surrounding the strands were motile, aerobic bacteria. After a few minutes, the bacteria had congregated around the portions of the filament illuminated by red and blue light. Assuming that the bacteria were congregating in regions where oxygen was being evolved in photosynthesis, Engelmann concluded that red and blue light are the most effective colours for photosynthesis. Below are diagrams of an action and absorption spectrum. The action spectrum shows which wavelengths of light are most used in the process of photosynthesis. The absorption spectrum shows the wavelengths of light which are most absorbed by the plant.

Action spectrum



Absorption spectrum



The light wavelength will be controlled during the experiment by using the same lamp at the same distance from the *Elodea* each time. The lamp will be plain 'white' light with no coloured filter.

- **Enzyme concentration** – Reactions in the light independent reaction are catalysed by enzymes. In this case, rubisco is the enzyme and RuBP and carbon dioxide are the substrates. At low concentrations of the enzyme, there are more substrate molecules than enzyme molecules. This means that all the active sites are filled with substrate molecules, and there are substrate molecules 'waiting' for an empty active site to bind with, so there will be a fairly low rate of reaction. At higher concentrations, there are more active sites available, so there are more catalytic events/second, so the rate of reaction will increase. Doubling the number

of enzyme molecules will double the number of active sites available for the substrate to bind with, therefore a doubling of the rate of reaction up to a certain point. However, as higher concentrations of enzyme are used, over half the substrate molecules will be bound to enzymes, so the rate of reaction will not double with enzyme concentration, but the reaction will still speed up. At a certain point of enzyme concentration, the reaction will not get faster, even if more enzyme is added, because all the substrate molecules will be bound to the active sites of enzymes. The graph to show the effect of varying enzyme concentration on an enzyme controlled reaction is as follows:

(Cambridge Biology 1)

Internal factor:

- **Concentration of photosynthetic pigments** – The greater the concentration of photosynthetic pigments the greater the amount of light that can be absorbed. This has an affect on the rate of photosynthesis in the same way as light intensity, as the pigments absorb the light utilised in photosynthesis, so the more photosynthetic pigments the more light absorbed and therefore the faster the rate of photosynthesis. This factor will be controlled by using the same piece of *Elodea* to ensure the concentration of photosynthetic pigments will be exactly the same. The exact length of *Elodea* to be used will be determined in the preliminary investigation.

In order for this experiment to be fair, all variables except the variable to be investigated must be kept constant. Temperature is the variable being investigated so all the others have to be kept constant. This will be done in the following ways:

Prediction

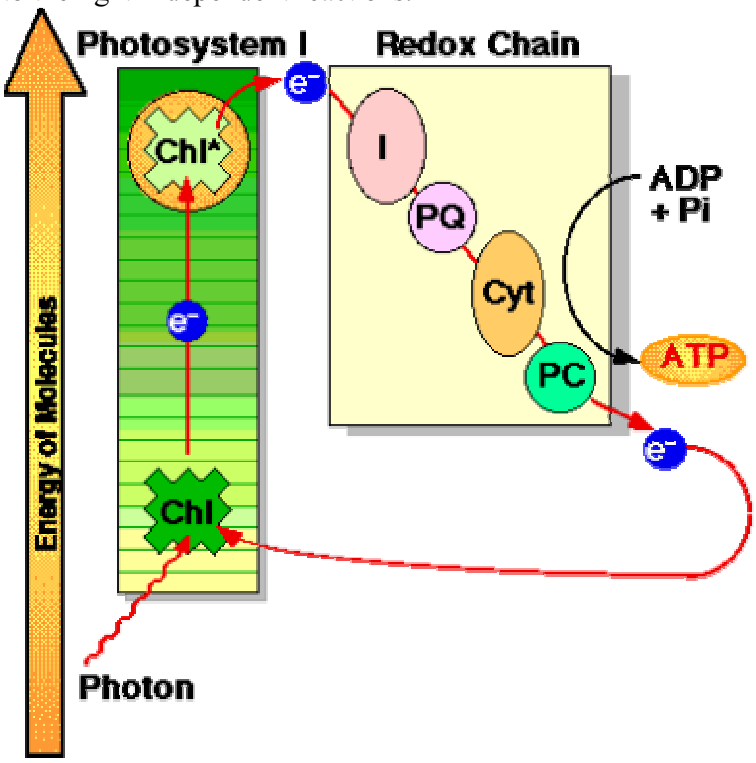
I predict that the rate of reaction of temperature will increase up to a maximum rate of about 30^0C. In addition I predict that the rate will double with every rise of 10^0C. After the maximum rate, the rate will start to decrease more rapidly, and the gradient of the decrease will be steeper than that of the increase. I predict that photosynthesis will no longer take place at about 50^0C and the graph will reach and stay at a rate of 0, even if the temperature is further increased.

Hypothesis

First, it is necessary to discuss the light-dependant and light-independent stages of photosynthesis.

Light-dependant stage – These reactions include the synthesis of ATP in photophosphorylation and the breakdown of water by photolysis to produce hydrogen ions. The hydrogen ions combine with NADP to produce reduced NADP. *ATP and reduced NADP pass from the light dependant stage of photosynthesis to the light independent stage.* Photophosphorylation of ADP to ATP can be either cyclic or non-cyclic depending on the patter of electron flow in one or both of the photosystems.

Cyclic photophosphorylation only involves photosystem I. Light is absorbed by photosystem I and is passed to chlorophyll a (P700). An electron in the chlorophyll a molecule is excited to a higher energy level and is emitted from the chlorophyll molecule. Instead of falling back into the photosystem and losing its energy as fluorescence, it is captured by an electron acceptor and passed to a chlorophyll a (P700) molecule via a chain of electron carriers. During this process enough energy is released to synthesise ATP from ADP and an inorganic phosphate group. The ATP then passes to the light-independent reactions.



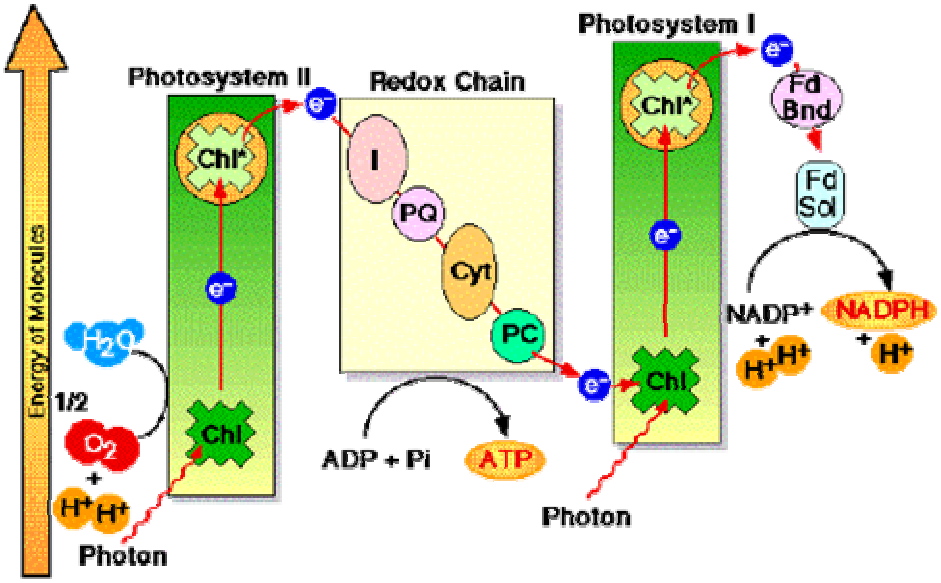
Non-cyclic photophosphorylation involves both photosystems in the 'Z scheme' of electron flow. Light is absorbed by both photosystems and excited electrons are emitted from the primary pigments of both reaction centres (P680 and P700). These electrons are absorbed by electron acceptors and pass along chains of electron carriers leaving the photosystems positively charged. These electrons need to be replaced

immediately so that the pigment can be returned to its stable state. For this reason, water is photolysed producing two electrons as shown below:

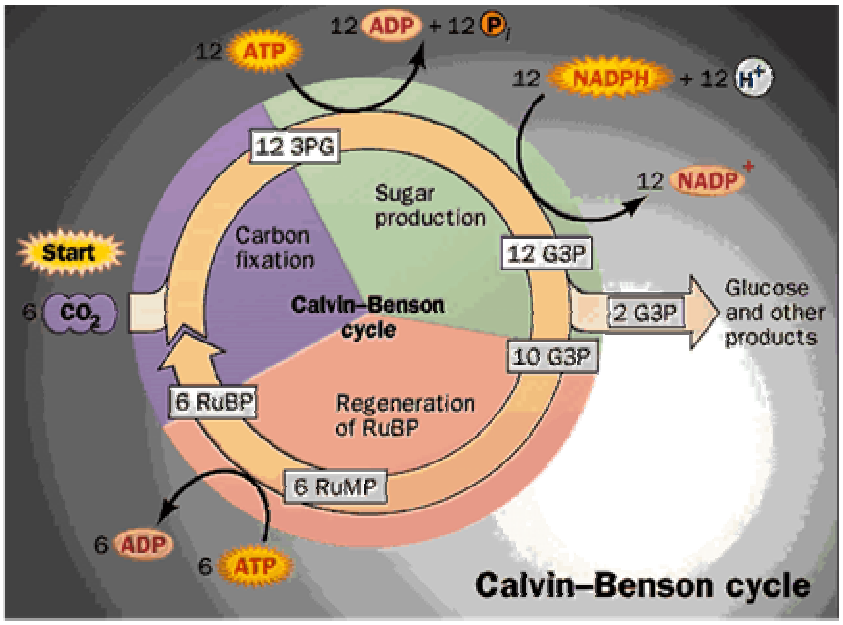
$$H_2O \longrightarrow 2H^+ + O_2 + 2e^-$$

The oxygen produced from the photolysis is a waste product of photosynthesis and is not needed for any reactions in the plant. For this reason the oxygen is emitted as a gas. The P700 of photosystem I absorbs electrons from photosystem II. P680 receives replacement electrons from the photolysis of water. ATP is synthesised as the electrons lose energy whilst passing along the carrier chain, as in cyclic photophosphorylation.

Below is a diagram of the 'Z scheme' of electon flow in photophosphorylation:

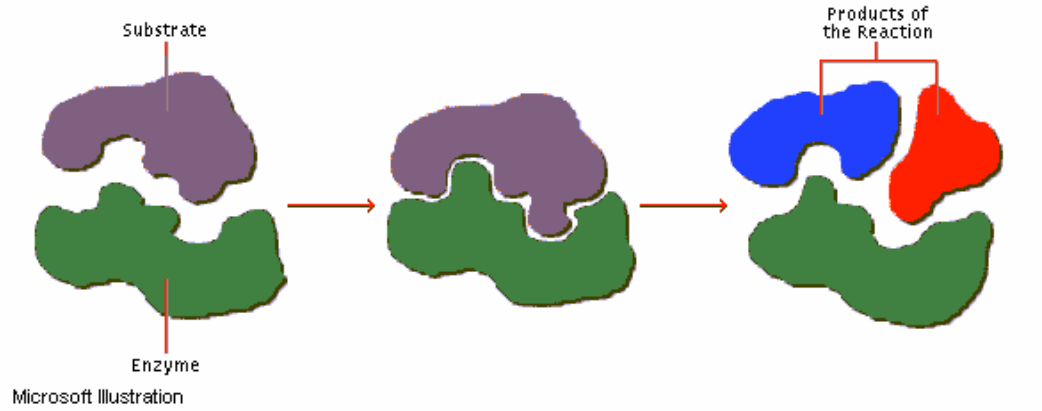


Light-independent stage – The fixation of carbon dioxide is a light-independent process in which carbon dioxide combines with RuBP (ribulose bisphosphate), to give two molecules of GP (glycerate 3-phosphate). This fixation is controlled by the enzyme RuBisCo. This stage is said to be thermochemical because the reaction is directly affected by temperature. This is because enzymes are affected by temperature, as explained later on. In the prescence of ATP and reduced NADP, produced in the light dependant stage, GP is reduced to triose phosphate. At this point carbohydrate is produced. Some of the triose phosphates condense to produce hexose phosphates, sucrose, starch and cellulose, or are converted to acetylcoenzyme A to make amino acids and lipids, to be used for other functions within the plant. Other triose phosphate molecules regenerate RuBP. This stage is usually called the Calvin cycle, which is shown below:



The reason why temperature has any effect on the rate of photosynthesis is due to enzyme action. Temperature has a large effect on the functioning of enzymes. There are two different explanations for the functioning of enzymes, one simple and one slightly more complex.

The more simple of the two is the 'lock and key' hypothesis. An enzyme is a globular protein, coiled into a precise 3D shape. This has an active site, which is a depression into which another molecule can bind. This molecule is the substrate of an enzyme, as it has a complementary shape which fits into the shape of the active site perfectly. Each type of enzyme will usually only act on one type of substrate molecule, as the shape of the active site will usually only allow one shape of molecule to fit in. For this reason the enzyme in said to be specific for this substrate. The enzyme then catalyses a reaction into which the substrate molecule is split into two or more molecules, called the products. A diagram of this is shown below:



However, the lock and key hypothesis is over-simplified, and not exactly how the process takes place. The more accurate hypothesis is the 'induced fit' hypothesis. This hypothesis is more based around the protein nature of the enzyme, allowing for flexibility and for the shape of the active site to be altered slightly. The binding of the enzyme and the substrate molecule because of the attraction between groups on the

substrate molecule and complementary groups on the active site of the enzyme molecule. The substrate molecule does not fit perfectly with the active site, rather forces its way in and in doing so alters the shape of the active site, thus a better more tight fit. Once the products have been released, the enzyme and active site returns to its original shape.

The main reason for enzyme controlled reactions is to lower the activation energy of a reaction. As catalysts, enzymes increase the rate at which chemical reactions occur. Most of the reactions which occur in living cells would occur so slowly without enzymes that they would virtually not happen at all. In many reactions, the substrate will not be converted to a product unless it is given some extra energy. This is called the activation energy. One of the main ways to give a activation energy is to heat it. Enzymes reduce the activation energy of the reactions they catalyse. They do this by holding the substrate or substrates in such a way that their molecules can react much more easily.

Enzymes also work on the basis of kinetic theory. In order that enzyme and substrate molecules bind, they must collide with the right amount of energy and at the correct angle. Therefore if the molecules have more kinetic energy, such as from heat, there will be more collisions because the molecules are increased in speed, therefore and increase in rate of reaction. In the experiment, a higher temperature means a higher kinetic energy and therefore faster rate of fixation of RuBP and carbon dioxide catalysed by RuBisCo to produce GP. GP is then reduced to triose phosphate in the presence of ATP and reduced NADP. ATP and reduced NADP are produced in the light dependant reaction. In high temperatures, to keep up with the high demand of ATP and reduced NADP, the light dependant reaction needs to take place quickly, relying on a lot of light. If there is a lot of light, the light dependant reaction takes place as quickly as the other limiting factors involved allow. This means a great demand for electrons from photolysis, so photolysis takes place at a faster rate than at lower temperatures, thus producing more oxygen per time as a waste product. Other factors involved in enzyme controlled reactions are inhibitors and activators, but neither occur in this reaction so the explanation of these factors is unnecessary.

The reason why I have predicted a downwards curve after the maximum rate is due to the denaturising of enzymes. Above the temperature of the maximum rate the structure of the enzyme molecule vibrates so energetically that some of the bonds holding the enzyme molecule in its precise shape begin to break, especially the hydrogen bonds. The enzyme molecule begins to lose its shape and activity and is said to be denatured. This is usually irreversible, so even a reduction in temperature would not cause the enzyme to function again.

Preliminary Investigation

For this investigation it is necessary to keep all variables except temperature constant. For the experiment to be done effectively, the point at which the variables are fixed needs to be decided. Two test runs of the experiment, but this time varying carbon dioxide concentration and light intensity, were done to find answers to a number of questions:

- What length of Elodea should be used?

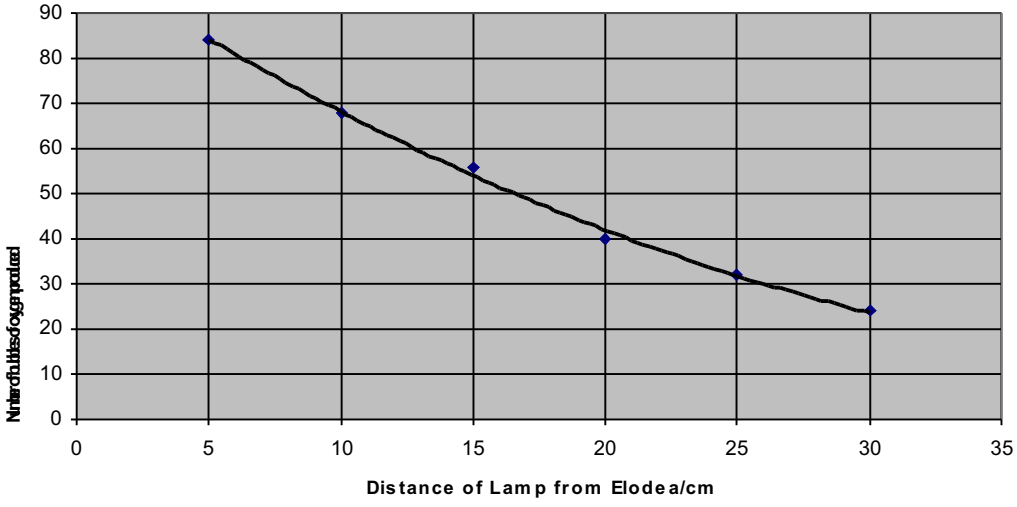
- How many readings should be taken in order to achieve reliable results?
- What apparatus should be used to produce the most accurate results?
- What concentration of hydrogen carbonate should be used?
- How far away should the lamp be positioned?
- What range of temperature readings should be used in order to support a firm conclusion?
- What is the best way to measure results?

The first preliminary experiment was done to investigate the effect of light intensity on the rate of photosynthesis. The aim of this experiment was to discover the distance the lamp needed to be from the elodea. This experiment was carried out in the same way as the method described below, but this time varying the distance the lamp was from the elodea as opposed to the temperature. This experiment was measuring the amount of oxygen produced in 30 seconds. The results were as follows:

Table 1: Table to Show Rate of Production of Oxygen at Different Light Intensities

Lamp distance from elodea (cm)	Number of bubbles
5	84
10	68
15	56
20	40
25	32
30	24

Here is a small graph of the results above:



This graph shows that as the distance from the lamp increases, the number of bubbles decreases, so the relationship between the two variables is inversely proportional.

The second trial experiment was to investigate the effect of carbon dioxide concentration on the rate of photosynthesis. This was done by varying the strength of the hydrogen carbonate solution. This time, instead of measuring the number of bubbles, a capillary tube was used and the oxygen collected to produce one large bubble. This bubble was then measured and the volume worked out. The results below shows the how the length of the bubble varies with varying concentrations of hydrogen carbonate solution:

The results from the trial experiments and graphs provided an indication of the answers to the questions posed earlier.

- The Elodea should be approximately 10cm long
- In order to achieve reliable results, each reading should be repeated preferably 3 times, with the results averaged.
- The apparatus used will be explained later on
- 1.5% hydrogen carbonate solution will be used
- The lamp will be positioned 10cm from the Elodea
- The temperature will start at 5^0C and will be increased in 10^0C steps up to the point where no more gas is produced
- The best way to measure the results is by letting the gas collect as one bubble and then measure the bubble.

Apparatus

- Elodea
- Table lamp
- Water bath
- Ice
- Bunsen burner
- Car
- 'S' bend capillary tube
- Test tubes
- Sodium hydrogen carbonate solution (1.5%)
- Distilled water
- Stop clock
- 30cm ruler
- Clamp stand and boss

Method

- The apparatus will be set up as shown in the diagram on the previous page.
- The water bath will be set at a certain temperature. Readings will be taken at the following temperatures: 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 35^0C, 40^0C, 45^0C and 50^0C.
- To set the temperature of the water bath below room temperature, ice will be added to the water bath and the temperature of the water measured with a thermometer until the temperature is correct. The thermometer will be read at eye level.
- To set the temperature of the water bath above room temperature, hot water will be added to the water bath and the temperature of the water measured with a thermometer until the temperature is correct.
- The hydrogen carbonate solution will be in the water bath as it is heated so it is at the desired temperature for the experiment.
- The stem of the *Elodea* is pushed a short way into the capillary tube.
- All the leaves of the *Elodea* are then submerged in the hydrogen carbonate solution and left to come to equilibrium.
- The lamp is position 10cm away from the *Elodea* and switched on.
- The experiment is kept as far away as possible from other peoples experiment so as to minimise the amount of stray light affecting the experiment.
- At this point the stop clock is started in order to measure 2 minutes.
- At 2 minutes, the *Elodea* is pulled from the capillary tube.
- The bubble at the bottom of the capillary tube is pulled up by the syringe.
- The length of the bubble is measured with the capillary tube at eye level.
- This method is repeated for the range of temperatures as explained above.

Justification

- The bubble length will be measured as opposed to counting the number of bubbles given off in a certain time. The first reason for this is that if the plant has a very high rate of photosynthesis, it is extremely difficult to count the number of bubbles produced because too many are being produced in a short space of time. Another reason behind the choice is that the size of the bubbles is variable so bubbles are not an accurate way of deciding the relative volumes of gas given off at each temperature. If number of bubbles was the variable the results would be inaccurate as to the volume of oxygen produced.
- The *Elodea* will be 10cm long in order that it is fairly long so there are more leaves to photosynthesise, so as to produce quite a lot of gas, but also so all the leaves fit into the hydrogen carbonate solution so that all the leaves receive the same amount of carbon dioxide.
- The experiment should be repeated 3 times and averaged to ensure the experiment is as reliable as possible to ensure a conclusion can be firmly supported.
- The lamp will be positioned 10cm from the *Elodea.* This is because in the preliminary investigation, it was ascertained that the results for the lamp being 10cm away from the *Elodea* was easiest to work with, as not too much or too little gas was produced.
- 1.5% hydrogen carbonate solution will be used at a volume of $40cm^3$. This is because 1.5% produced a good amount of gas, again not too much or too little.

- The temperature will start at 5^0C and will be increased in 5^0C intervals up to 50^0C. The starting point is 5^0C because below this temperature, due to the nature of enzymes, very little or no gas will be produced, but a small amount of gas should be produced at 5^0C. 5^0C intervals will be used because it is then easy to calculate the Q10 values to see if the prediction is correct. The experiment will be carried out up to 50^0C because it is expected that no gas will be produced at this point because the enzymes will have been denatured.

- An 'S' shaped capillary tube will be used as opposed to a straight capillary tube. This is because with a straight tube, all the gas produced will travel straight up the capillary tube up to the syringe, meaning no bubble can be measured. With the bend in the tube, the gas will collect at the bend forming one bubble with all the gas produced. This can be drawn up by the syringe and the length of the bubble measured.

- All of the readings of the thermometer and the ruler against the bubble will be taken at eye level to ensure the value is read precisely to ensure the results are accurate.

Risk Assessment

The main danger of this experiment is the hot water. It is essential to be careful when holding a beaker full of the hot water to ensure none is spilt on the skin. If hot water is spilt on the skin then it will cause scalding. It is necessary to have cold water near the experiment in case hot water gets on the skin, to cool the skin off. The only other small risk is that some of the apparatus is glass, so care should be taken so as not to drop the glass, as shards can cut into the skin and can often cause a permanent scar.

Results

Table 1: Table to show how the length and volume of an oxygen bubble varies with temperature in 2 minutes

	Length of oxygen bubble (mm per 2 mins)				
Temperature (°C)	**1**	**2**	**3**	**ave**	Volume of oxygen bubble (mm^3min-1)
5	1	2	2	2	3.14
10	7	9	7	8	12.6
15	**19**	12	14	13	**23.6**
20	18	19	19	19	29.8
25	26	25	27	**26**	**40.8**
30	**24**	45	43	44	69.1
35	38	37	40	38	59.7
40	28	30	30	29	45.6
45	8	7	8	8	12.6
50	0	0	0	0	0

The results underlined and in bold are anomalous results as they are deemed to far from the other values recorded to be accurate.

Analysis

The graph shows that as the temperature increases up to a point, so does the amount of oxygen produced. From this maximum point, the amount of oxygen produced decreases with temperature up to the point where no more oxygen is produced. The graph shows that there is a fairly steady increase in rate between 5^0C and 30^0C. 30^0C is the temperature at which most oxygen is produced in this experiment. On the graph, the gradient is steeper in decrease than in increase. From the results it is possible to plot some Q10 values, to determine whether for every 10^0C rise, the rate of photosynthesis doubles.

1. 10^0C to 20^0C – 29.8/12.6 = 2.4
2. 15^0C to 25^0C - 40.8/23.6 = 1.7
3. 20^0C to 30^0C - 69.1/29.8 = 2.3

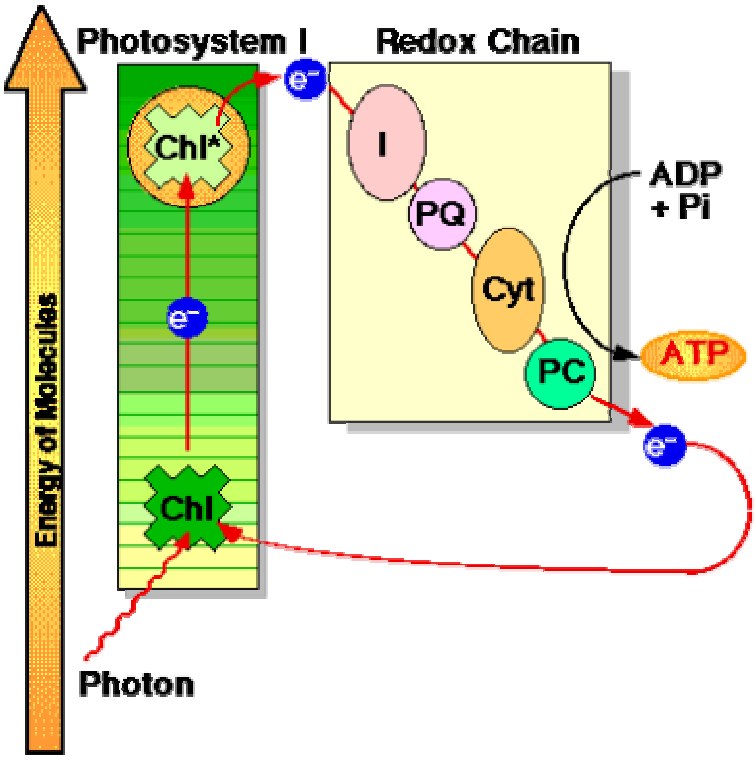
Although the readings at 25^0C were deemed anomalous, they were only slightly too low. If they were a little bit higher as expected the Q10 value would be almost exactly 2.

The original prediction was*: 'I predict that the rate of reaction of temperature will increase up to a maximum rate of about 30 ^{0}C. In addition I predict that the rate will double with every rise of 10^{0}C. After the maximum rate, the rate will start to decrease more rapidly, and the gradient of the decrease will be steeper than that of the increase. I predict that photosynthesis will no longer take place at about 50 ^{0}C and the graph will reach and s tay at a rate of 0, even if the temperature is further increased*.'

From the results obtained it is fair to say that the prediction was accurate. It is now necessary to look at the way in which temperature affects oxygen production. First, it is necessary to discuss the light-dependant and light-independent stages of photosynthesis.

Light-dependant stage – These reactions include the synthesis of ATP in photophosphorylation and the breakdown of water by photolysis to produce hydrogen ions. The hydrogen ions combine with NADP to produce reduced NADP. *ATP and reduced NADP pass from the light dependant stage of photosynthesis to the light independent stage.* Photophosphorylation of ADP to ATP can be either cyclic or non-cyclic depending on the patter of electron flow in one or both of the photosystems.

Cyclic photophosphorylation only involves photosystem I. Light is absorbed by photosystem I and is passed to chlorophyll a (P700). An electron in the chlorophyll a molecule is excited to a higher energy level and is emitted from the chlorophyll molecule. Instead of falling back into the photosystem and losing its energy as fluorescence, it is captured by an electron acceptor and passed to a chlorophyll a (P700) molecule via a chain of electron carriers. During this process enough energy is released to synthesise ATP from ADP and an inorganic phosphate group. The ATP then passes to the light-independent reactions.

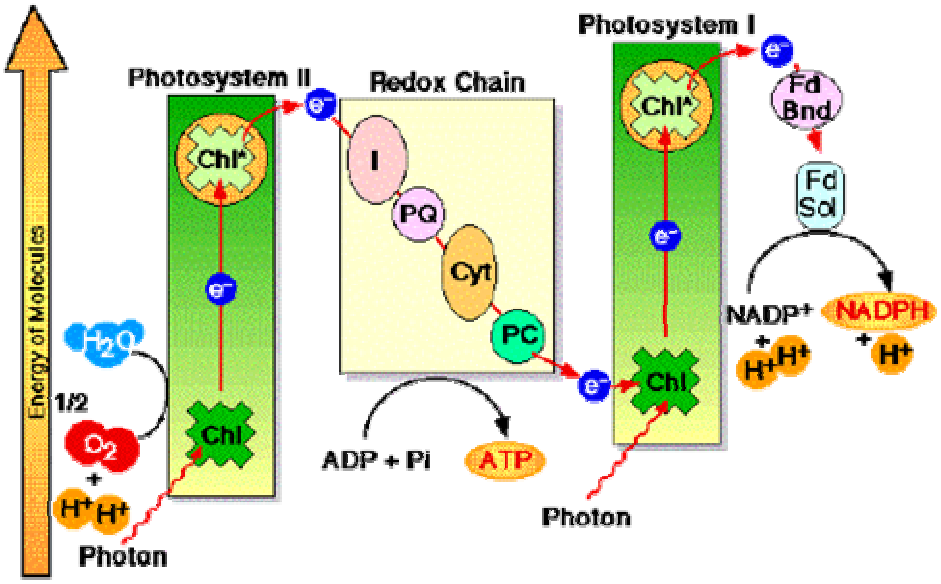


Non-cyclic photophosphorylation involves both photosystems in the 'Z scheme' of electron flow. Light is absorbed by both photosystems and excited electrons are emitted from the primary pigments of both reaction centres (P680 and P700). These electrons are absorbed by electron acceptors and pass along chains of electron carriers leaving the photosystems positively charged. These electrons need to be replaced immediately so that the pigment can be returned to its stable state. For this reason, water is photolysed producing two electrons as shown below:

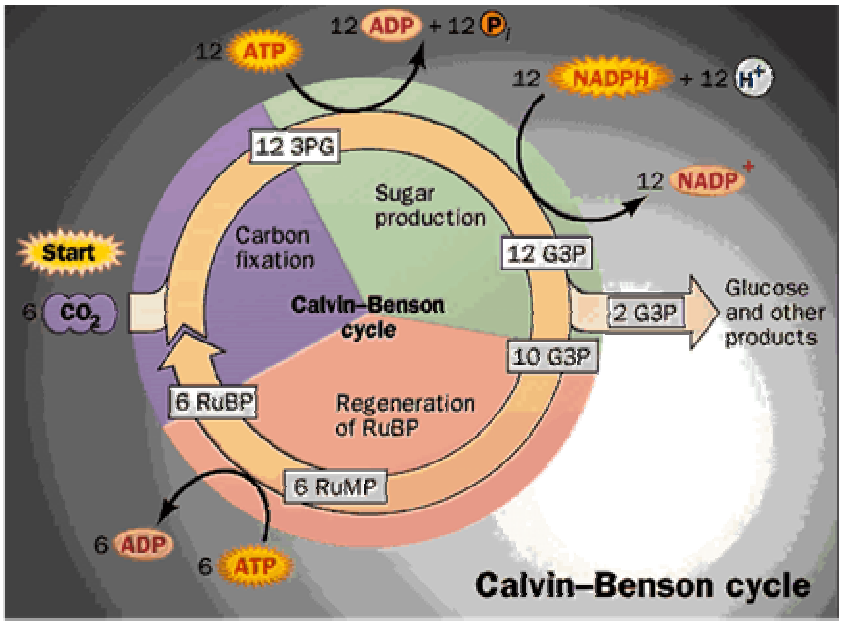
$$H_2O \longrightarrow 2H^+ + O_2 + 2e^-$$

The oxygen produced from the photolysis is a waste product of photosynthesis and is not needed for any reactions in the plant. For this reason the oxygen is emitted as a gas. The P700 of photosystem I absorbs electrons from photosystem II. P680 receives replacement electrons from the photolysis of water. ATP is synthesised as the electrons lose energy whilst passing along the carrier chain, as in cyclic photophosphorylation.

Below is a diagram of the 'Z scheme' of electon flow in photophosphorylation:

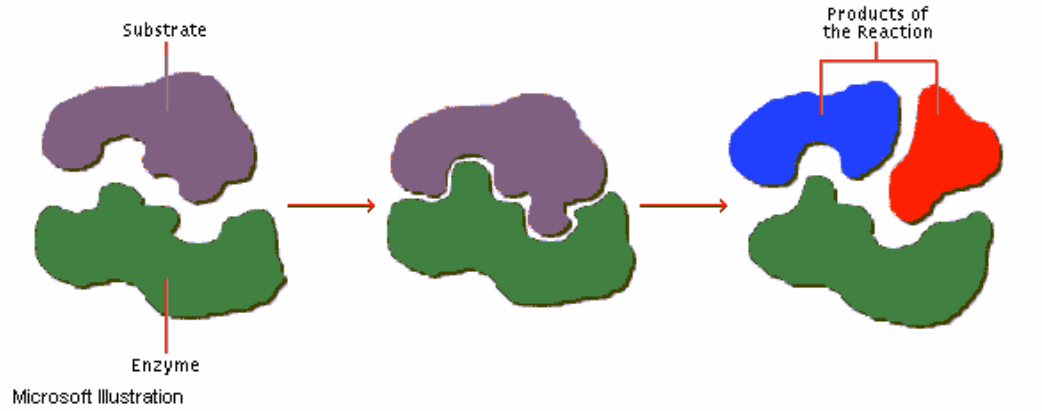


Light-independent stage – The fixation of carbon dioxide is a light-independent process in which carbon dioxide combines with RuBP (ribulose bisphosphate), to give two molecules of GP (glycerate 3-phosphate). This fixation is controlled by the enzyme RuBisCo. This stage is said to be thermochemical because the reaction is directly affected by temperature. This is because enzymes are affected by temperature, as explained later on. In the prescence of ATP and reduced NADP, produced in the light dependant stage, GP is reduced to triose phosphate. At this point carbohydrate is produced. Some of the triose phosphates condense to produce hexose phosphates, sucrose, starch and cellulose, or are converted to acetylcoenzyme A to make amino acids and lipids, to be used for other functions within the plant. Other triose phosphate molecules regenerate RuBP. This stage is usually called the Calvin cycle, which is shown below:



The reason why temperature has any effect on the rate of photosynthesis is due to enzyme action. Temperature has a large effect on the functioning of enzymes. There are two different explanations for the functioning of enzymes, one simple and one slightly more complex.

The more simple of the two is the 'lock and key' hypothesis. An enzyme is a globular protein, coiled into a precise 3D shape. This has an active site, which is a depression into which another molecule can bind. This molecule is the substrate of an enzyme, as it has a complementary shape which fits into the shape of the active site perfectly. Each type of enzyme will usually only act on one type of substrate molecule, as the shape of the active site will usually only allow one shape of molecule to fit in. For this reason the enzyme in said to be specific for this substrate. The enzyme then catalyses a reaction into which the substrate molecule is split into two or more molecules, called the products. A diagram of this is shown below:



However, the lock and key hypothesis is over-simplified, and not exactly how the process takes place. The more accurate hypothesis is the 'induced fit' hypothesis. This hypothesis is more based around the protein nature of the enzyme, allowing for flexibility and for the shape of the active site to be altered slightly. The binding of the enzyme and the substrate molecule because of the attraction between groups on the

substrate molecule and complementary groups on the active site of the enzyme molecule. The substrate molecule does not fit perfectly with the active site, rather forces its way in and in doing so alters the shape of the active site, thus a better more tight fit. Once the products have been released, the enzyme and active site returns to its original shape.

Enzymes work on the basis of kinetic theory. In order that enzyme and substrate molecules bind, they must collide with the right amount of energy and at the correct angle. Therefore if the molecules have more kinetic energy, such as from heat, there will be more collisions because the molecules are increased in speed, therefore and increase in rate of reaction. In the experiment, a higher temperature means a higher kinetic energy and therefore faster rate of fixation of RuBP and carbon dioxide catalysed by RuBisCo to produce GP. GP is then reduced to triose phosphate in the presence of ATP and reduced NADP. ATP and reduced NADP are produced in the light dependant reaction. In high temperatures, to keep up with the high demand of ATP and reduced NADP, the light dependant reaction needs to take place quickly, relying on a lot of light. If there is a lot of light, the light dependant reaction takes place as quickly as the other limiting factors involved allow. This means a great demand for electrons from photolysis, so photolysis takes place at a faster rate than at lower temperatures, thus producing more oxygen per time as a waste product. Other factors involved in enzyme controlled reactions are inhibitors and activators, but neither occur in this reaction so the explanation of these factors is unnecessary.

The reason the graph started to curve downwards fairly rapidly after the temperature at which maximum rate occurred is due to the denaturing of enzymes. Above the temperature of the maximum rate the structure of the enzyme molecule vibrates so energetically that some of the bonds holding the enzyme molecule in its precise shape begin to break, especially the hydrogen bonds. The enzyme molecule begins to lose its shape and activity and is said to be denatured. This is usually irreversible, so even a reduction in temperature would not cause the enzyme to function again.

Evaluation

The results obtained showed a clear pattern and allowed for a firm conclusion to be made, so it is ascertained that the method was suitable for the experiment. However the experiment was certainly not perfect as there were anomalous results and improvements to certain parts of the method could be made to reduce the likelihood of errors.

Method Evaluation

Starting from the beginning of the experiment, the first possible error is in the making up of the hydrogen carbonate solution. Due to human errors and misuse of equipment the percentage of the solution may not have been entirely correct. Syringes with a capacity as close to the amount of hydrogen carbon solution needed as possible in order that the volume of hydrogen carbonate solution be measured as accurately as possible. The same can be said for water in the mixing of these two substances. However syringes are not the most accurate way of measuring out volumes of liquid. For this a burette should be used as they have been constructed to a greater degree of accuracy. However, for this experiment a burette would have been impractical as there was not enough time allocated for the experiment. It was decided that it was better to use the syringes and have a slightly less degree of accuracy but in using syringes a greater number of readings could be taken. The percentage error of the syringes used for the small volumes taken was extremely small, so syringes were more practical to use in this experiment.

One of the largest sources of error in this experiment was temperature control. It is extremely to keep the temperature constant for three readings using just ice and hot water. Also, the lamp was extremely close to the water bath, which was made of glass, so the light would have been magnified into the water, and therefore heating the water. The most effective way of controlling the temperature of the experiment is to use a thermostatically controlled water bath. This would mean the temperature could be kept entirely accurate, and therefore increasing the accuracy and reliability of the experiment. As the difference between two readings taken was only 5^0C, any inaccuracy in temperature would mean a big difference in the gap between the two results. In an ideal situation, the thermostatically controlled water bath would be used as it both increases accuracy and saves time. However the experiment was carried out as precisely as possible and the temperatures kept to the desired value as much as possible.

Another major source of error in this experiment was the light intensity. The blinds were close and the lights turned off to minimise the amount of extra light in the room. However other people had lamps on to do their experiment so there was a lot of stray light which would increase the rate of photosynthesis. This would not have been such a big problem had the light intensity stayed constant. However people were switching their lamps on and off meaning the light intensity varied slightly throughout this experiment. It is too difficult to know how much of an effect this had. One possible solution is to be put a black screen around the experiment. However this would be impractical as it would be impossible to take readings at eye level. The best solution would be to do the experiment alone in a room with no windows so the light intensity would be kept constant throughout the experiment.

Measurement is another source of error. It is very difficult to take an extremely accurate measurement of the length of the bubble by holding the ruler up next to the tube. Rulers have an accuracy of +/-0.5mm. With the smallest readings, such as 2mm, this meant the results taken was extremely inaccurate. There is no real improvement than can be made to this as a ruler is the only way of measuring the length of the bubble. The only was of getting accurate measurements is by being extremely careful to take the readings precisely.

Another problem with the experiment could have been the *Elodea* being forced to photosynthesise too much. This could have damaged the plant, more likely at the higher temperatures, and therefore subsequent readings may not demonstrate a true pattern of the rate of photosynthesis at different temperatures. One way to combat such problem is to use a different piece of *Elodea* for each reading. However, this would not be a fair test as the different bits of *Elodea* would have different surface areas and slightly different concentrations of chloroplasts.

Doing the experiment alone was practically difficult in that it was difficult to ensure each reading was taken after exactly 2 minutes. The *Elodea* was to be placed in the hydrogen carbonate solution and the stop clock started immediately, which is an impossible task for one person. One way of rectifying this problem is to have one person to do the experiment and another to do the timing. However, the errors caused by the timing would be very minor and relatively insignificant so the method used was most practical.

Results Evaluation

Table 1: Table to show how the length and volume of an oxygen bubble varies with temperature in 2 minutes

	Length of oxygen bubble (mm per 2 mins)				
Temperature (°C)	**1**	**2**	**3**	**ave**	Volume of oxygen bubble ($mm^3 min^{-1}$)
5	1	2	2	2	3.14
10	7	9	7	8	12.6
15	**19**	14	12	13	**23.6**
20	18	19	19	19	29.8
25	26	25	27	**26**	**40.8**
30	**24**	45	43	44	69.1
35	38	37	40	38	59.7
40	28	30	30	29	45.6
45	8	7	8	8	12.6
50	0	0	0	0	0

The results underlined and in bold are anomalous results as they are deemed to far from the other values recorded to be accurate.

The anomalies from column 1 (first readings) will be explained first, with their percentage errors, but separately as there are different explanations for each anomaly.

Percentage Error

15°C [(19-13)/13]*100 = 46%

25°C [(24-44)/44]*100 = 45%

The first of these anomalies has a very large percentage error. This error is unacceptably high as it is not close enough to the other readings to be regarded when calculating the average. Although the percentage error is high, the average value of oxygen produced was fairly low so the anomalous reading is not too far out. The mostly likely possibility for this anomaly is that the temperature was too high as it was the first reading taken at 15°C and the water temperature was too high. Due to human error in not allowing the temperature of the water to even out through the whole beaker the temperature was seen to be 15°C on the thermometer. This meant the *Elodea* had a higher rate of photosynthesis and therefore more oxygen was produced. Ideally there would be more time in which to carry out the experiment so the water could be left to even out to the desired temperature. Even better, as explained above, would be to use a thermostatically controlled water bath.

The second of the above readings also had a very large percentage error. As the average amount of oxygen produced at 25°C was relatively high the difference is amounts produced between the average and the anomaly was also very high. The anomaly was almost certainly caused due to human error. Because the reading was so low, the most likely possibility was that the *Elodea* was not properly inserted into the capillary tube, and therefore some of the oxygen produced escaped into the water beaker. Temperature may have been a factor in that the temperature was not high enough, but as the anomaly was so far out from the average this is an unlikely explanation.

The two anomalies from the averages column are said to be anomalous because they do not lie on the line of best fit on the graph. However these points are not very far off the line and do not cause a problem is seeing the pattern from the graph. The point at 15°C on the graph was slightly too high. This could have been because the temperature was too high so the *Elodea* photosynthesised at a higher rate than it would at 15°C. The possible reason for the temperature difference has been explained above. Also at this point, there may have been a greater amount of stray light reaching the *Elodea* so the light intensity increased, increasing the rate of photosynthesis. However this is an unlikely possibility as the amount of stray light seemed to stay approximately the same throughout the experiment. However the light intensity was an immeasurable quantity so it is not possible to decide exactly how much of an affect it had.

The point at 25°C on the graph was slightly to low. This could have been due to gas escaping as previously explained. The temperature may have been slightly too low as the water strayed towards room temperature. This would cause a decrease in the rate in photosynthesis of *Elodea*. Also the light intensity may have been slightly less than the average throughout the experiment but as mentioned above this is an immeasurable quantity.

All of the improvements suggested for this experiment would undoubtedly improve the accuracy of the experiment and thus the reliability of the conclusions. However the graph shows a clear pattern of results and allows for a firm conclusion to be made. The results are accurate enough to make a reliable conclusion, so it can be said that the method used for this experiment was suitable.