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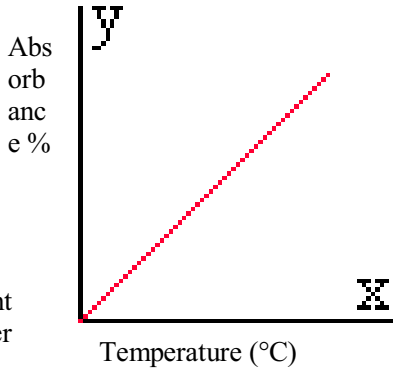
The effect of Temperature on Beetroot Membranes

Introduction:
All multi cellular cells are made up of a number of organelles. To function properly, a cell needs to be able to control the transport of substances, and this is done through a *selectively permeable membrane (8-10 micrometers thick*.) Beetroot contains red pigments that are found within the cell vacuole. Generally, the red pigment cannot pass through the cell membrane unless the cell is damaged, and in this case, the cell bleeds from the cell. The amount the beetroot 'bleeds' from a sample is an indication of the degree of damage. The following experiment can establish what effect an increase in temperature has on the plasma membrane.

Aim:
The aim of the investigation is to find out how an increase in surrounding temperature affects the activity of the plasma cell membrane of beetroot cells.

Hypothesis:
I predict that the beetroot will 'bleed' more dye as the surrounding temperature increases. This is because I expect that higher temperatures will distort and denature the 'active site' and therefore will effect the shape of the fluid mosaic model membrane. The increase of kinetic energy, (through an increased temperature) will speed up the diffusion rate of the red pigment (betalains) and then due to the damage of the membrane and the denatured carrier proteins, this will increase the amount of dye that is 'bled' out of the cells resulting in a higher absorbance rate.



Variables:

Type of variable:		
Independent	Temperature – measured in °C.	I will be changing the temperature by placing the different test tubes in different water baths. I will use the following temperatures: 0, 20,40,60 and 80 degrees.
Dependent	The percentage of absorbance (%)	I will do this by using a colorimeter, as this will show me the percentage of absorbance in the samples. The dependent variable will be recorded using quantitative data instead of qualitative, this is because as we want to plot a graph, we cannot do that using qualitative data and

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		therefore must use the appropriate type of data.
Controlled	- Beetroot - Colorimeter and same filter (blue/green @ 490nm) - Amount of water in each test tube - Type of water (distilled) - Same length, size and surface area of beetroot sections - Keep the beetroot samples in the water for the same amount of time.	- Colorimeter filter will be kept and measured in (nm) and the volume of water will be kept to 5 cm (3) - I will use a blue/green filter, as it is a complimentary colour for the beetroot pigment. - It is important to keep the size of the beetroot cylinders the same as surface area is also a factor that effects the diffusion rate.

** It is important to keep the control variable the same as it makes the experiment as fair as possible, in addition to this, you won't be able to know if the changes to the independent variable are causing the changes to the dependent variable. I will keep them controlled by not changing the controlled variables I have mentioned. For example, I will get the same water from the same tap etc. so that there are no changes in the controlled variables.*

Apparatus:

- Stopwatch
- Colorimeter
- Variety of water baths
- Number of test tubes
- Beaker
- Cork borer
- White tile
- Knife
- Pipette
- Test tube rack
- Thermometer
- Ruler

Materials:

- Beetroot
- Distilled Water

Safety:

- Wear eye protection
- Beware of hot apparatus
- Take care with electricity supply
- *Be careful of beetroot dye

Method:

1) "Place 5 labeled test tubes each containing 5cm(3) distilled water into water baths at 0°c, 20°c, 40°c, 60°c and 80°c. Leave for at least 5 minutes until the water reaches the required temperature.
2) Cut sections from a single beetroot using a cork borer on a white tile. Cut five 1cm length slices from these sections.
3) Place the slices of beetroot in a beaker of distilled water and leave for 2 minutes to wash away the dye from cells damaged by the cork borer.

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4) Place one of the beetroot sections into each of the test tubes. Leave for 20 minutes in the water baths.
5) Decant the liquid from each tube, into a clean; labeled test tube ensuring the beetroot disc stays behind.
6) Switch the colorimeter and set it read % absorbance. Set the filter to the blue/green filter-490nm.
7) Measure 3cm(3) distilled water into a cuvette. Place the cuvette in the colorimeter ensuring that the light is shining through the clear sides. Zero the colorimeter (**R** button)
8) Measure 3cm(3) of one of the dye solutions into a colorimeter cuvette and take a reading absorbance (**T** button) repeat the readings for all the dye solutions."

Data Collection and Processing:

Table of results:

Independent variable	*Dependent Variable (+)*	*(+)*	*(+)*	*(+)*	*(+)*	*(+)*
Temperature/ (°c)	*Absorbance/ (%) Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*	*Sample 5*	*Average (for each temp)*
0	0.09	0.09	0.05	0.01	0.12	0.07
20	0.19	0.22	0.05	0.15	0.35	0.19
40	0.22	0.27	0.22	0.22	0.45	0.28
60	1.19	1.16	1.97	1.31	0.55	1.23
80	1.20	1.22	1.74	1.50	0.67	1.26

** Average calculation: (sample 1 + sample 2 + sample 3 + sample 4 + sample 5/5) for each temperature**

*Significant trend from results table: by looking at my results table, I can see that as the temperature increases, the average absorbance % increased to. Therefore they are directly proportional.

Standard Deviation:

Temperature	Standard Deviation	Standard deviation (/2) – as shown on graph	3 sig figs.
0	0.047871355	0.0239356775	0.02
20	0.126062154	0.063031077	0.06
40	0.099649385	0.0498246925	0.05
60	0.505648099	0.2528240495	0.25

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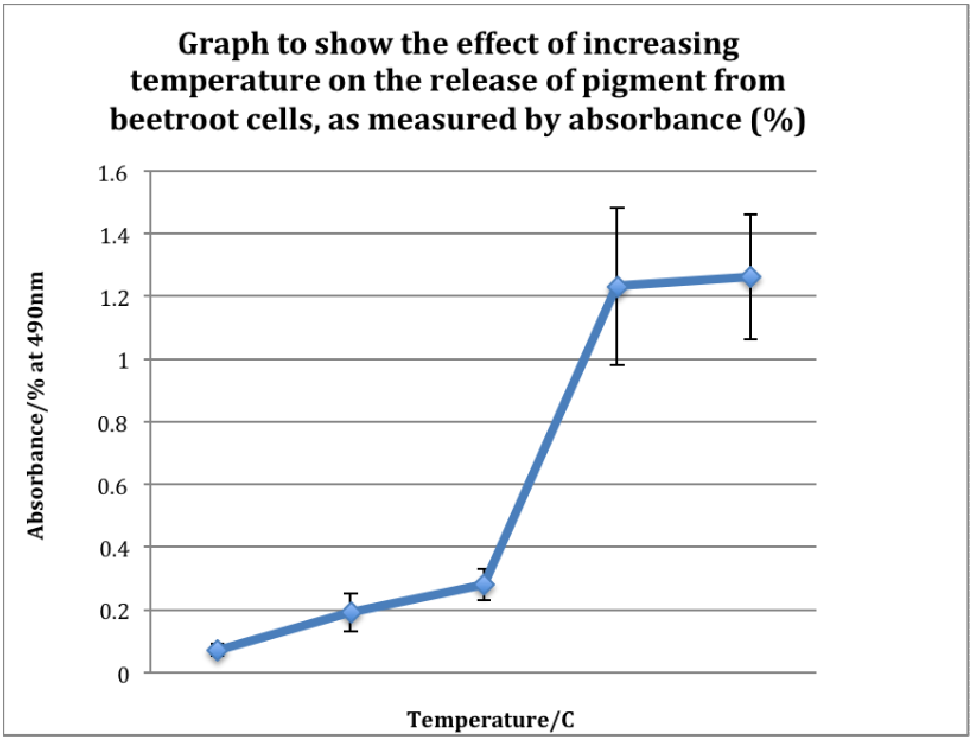
80	0.400349847	0.2001749235	0.20



Graph Analysis:
From my graph it is clear that as the temperature increased, the rate of absorbance also increased; directionally proportional. The temperature increased steadily, to an extent, however when it reached 40c, the absorbance percentage increased rapidly and started to level out at 60c. In addition to this, as my error bars show in both my graph and table, that not all my data was under 0.05 therefore I can not put the errors in my results down to chance and errors in my experiment, I will discuss later.

Conclusion:
I can conclude, from my graph, that there is a general trend in my results. The trend is that as the temperature increases the mean percentage of absorbance increased too. From temperatures 0-40 (C) there was a gradual increase however, as the graph shows, it increased rapidly from temperature 40 to 60 and then gradually leveled out between 60-80 degrees.
The graph clearly shows how an increase in temperature can affect the membrane activity (which is shown through the absorbance percentage using a colorimeter.) The steep incline shown on my graph (between 60-80 degrees) represents the point in which the protein in the membrane cells are becoming denatured (due to the increased temp) and allowing more of the red pigment to leave the cell. The cell membrane has a phospholipid layer, which is made up of fatty acids and when it is placed in a high temperature, the layer becomes more fluid and therefore becomes more delicate. When the carrier proteins in the cell membrane become denatured (the optimum

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temperature for enzymes is between 10-35 degrees approx.) they form holes and destroy the delicate layer in the membrane. This results in the beetroot pigment 'bleeding' through and any pigment in the membrane will now exit the cell. This is represented between 40 degrees and 80 degrees in relation to the absorbance level increasing.
With reference to my hypothesis, the increase in temperature will increase in the amount of kinetic energy as the water will be hotter and expand resulting in more vibrations will pass through the water (kinetic energy), which will result in an increase in the diffusion rate of the pigment. Once the temperature reaches above approx. 40 degrees, the cell membrane plasma will denature due to the surrounding temperature being too high and consequently the beetroot pigment in the cell membrane will eventually leak out. However, at lower temperatures, less damage was done to the cell membrane. This was done by smaller amounts of pigment diffusing through the cell membrane and therefore absorption percentage was lower.
In conclusion, the temperature affects the activity of the cell membrane as the temperature increases. This is due to the fact that the carrier proteins in the cell membrane denature with higher temperatures as it becomes too hot for them to function. The change involving the phospholipid layer structure also results in the membrane becoming less stable and therefore more of the beetroot pigment is able to escape the membrane in addition to more carrier proteins destroyed.
From my graph and error bars I am able to say that for 0 and 40 degrees, the possibility that our results are just chance are under 0.05% and therefore we can be certain that our results are reliable due to this small percentage. However when looking at the other error bars, the percentage of error is quite high so consequently, it is more likely that a number of errors or mistakes contributed to the results that I got.

Evaluation:
From my data, it was evident that my results were not as accurate as they could have been and there are a number of factors that could contribute to my level of accuracy and the results that I got. The fact that my results follow a genuine trend suggests that my data was not far off however compared to some of the other students, a few of my results were different by a large extent and this can be seen evidently in my data table.

Source of error:	*Explanation & Improvement:*
- The beetroot cylinders were not always cut accurately and to the size that they should have been at.	Even though I used a ruler, to improve this I could have perhaps made an accurate template and cut out beetroot cylinders equal to that size.
- The temperature in the water baths varied so therefore it was hard to keep a constant desired temperature.	An exact temperature could not be determined as the removal of the water baths meant that heat escaped from the baths. To improve this, I could have put the water baths in more of an enclosed space and checked the temperature every minute or at least frequently and when the thermometer was at the right temperature, I could have taken it out and did that sample then. This would enforce more accurate results.
- Shortage of time and so	This links to the second one as I think

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consequently my results and data, in my opinion, were a bit rushed I thought.	that if I had more time I could have done each temperature one after the other instead of all at the same time so I could focus on each of them and achieved more accurate data.
- Intervals of temperature taken; there was too much of a gap between them.	To have more precise results, I could have had a smaller gap between the temperatures. There was a 20 degree gap, which is quite a large gap, if I had gone up in 5 degrees then I am sure that I would have not only had more accurate results but more detailed too.
- The beetroot cylinders were pushed out with a cork borer so therefore the cell membranes could have been damaged.	This would be a hard error to try and improve, as this is the most convenient way to cut them out. Therefore when doing the experiment, it is important to be careful.
- The beetroot cylinders were taken from different areas of the beetroot.	This was done assuming that the pigment was the same in the beetroot, however the outside could be more ductile to protect the inside of the beetroot. To improve this, a number of beetroots could be used so that the same place is used each time therefore making it a fairer test.
- When the samples were removed from the test tubes, some of the sample could still be at the bottom of the test tube; ie the pigment.	This would be quite hard to try and improve however when transferring the sample from the test tube to the cuvette, it is possible to scrape and make sure all of the sample is out and poured into the cuvette.
- Test tubes may have been dirty and therefore affected the colour or transparence of the test tubes.	This would have been a minor error however to improve this I could wash the test tubes out before using them and dry them. In addition I could keep them in a dry clean place to stop dirt or anything that could effect their cleanliness from coming in.
- Taking the five samples from the other students in the class; could affect my data and results.	The fact that the class put all the data together meant that there was room for variety in the results as different people using different equipment each did their own experiment. This is a clear indication that the results were not as accurate as they could have been.
- The percentage for error bars appeared to be quite high for a number of my temperatures.	The smaller the percentage means that there is less variety within the data. Due to the standard deviation and error bars results it shows that the experiment may have encountered a few problems. To decrease the % of error bars or standard

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deviation, I could do more repeats of the experiment.

However, by taking an average, which I did with each sample I think that this can show a level of accuracy with my results as because I had 5 samples, I was able to add all the results together to find a mean of the data and then plot this on a graph. In addition to this, by using a control sample of water to test the colorimeter, it meant that I could prove my colorimeter was working properly as when tested, it gave the correct absorbance percentage. Other procedures I used to ensure that variation and errors were kept to a minimum were all the beetroot cylinders were cut with the cork borer and I used a stopwatch to ensure that all test tubes were kept in for the same amount of time.
If I had to do the experiment again, another variable I could measure, instead of temperature is how the PH affected the cell membrane activity.