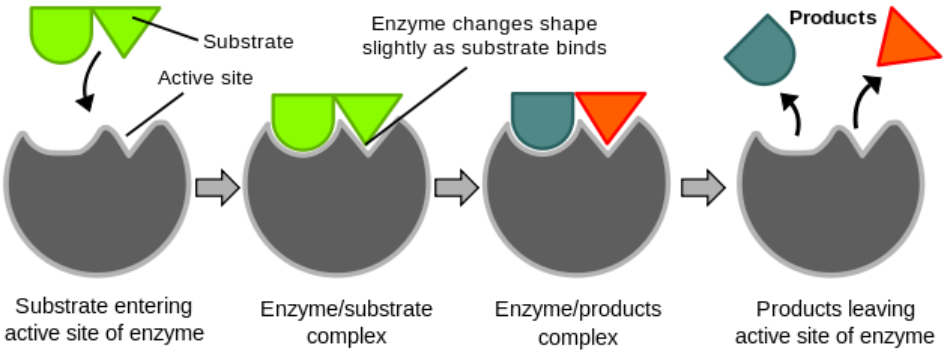
BIOLOGY

How does temperature affect enzyme activity?

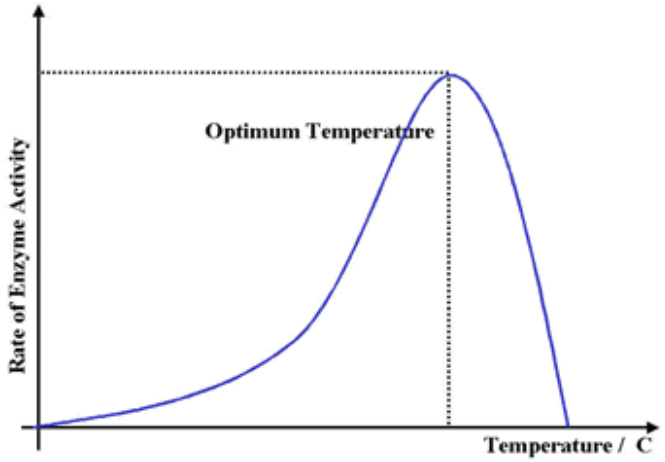
Introduction:

The sum of the thousands of living reactions that occur constantly in each living cell is called cellular metabolism. (Jones, 2016). To sustain life, organisms need to have a high cellular metabolism. This is achieved through enzymes.

Enzymes are protein biological catalysts that provide an alternate reaction pathway with a lower activation energy, allowing a reaction to proceed faster. Enzymes are specific for a particular substrate due to their active site, which is determined by the protein's tertiary structure. A complementary shaped substrate to the active site can bind to it, forming an enzyme-substrate complex. The enzyme then breaks the bonds in the substrate to release different products, as shown in Figure 1.



Enzymes have an optimal temperature at which they function. As the temperature of an environment increases, the kinetic energy within the molecules also increase, meaning they are more likely to react. However, if the temperature rises too high, it can denature the enzyme: permanently damaging the active site shape so the substrate can no longer bind. At very low temperatures, there is little kinetic energy in the molecules, so they collide less frequently and consequently have a lower reaction rate. If the temperature was increased again, however, the enzyme would function normally. Figure 2 shows a temperature-enzyme reaction rate graph.



Diastase is an enzyme group found in malt and in the pancreas that catabolically breaks down polysaccharide starch into glucose. Glucose is used for cellular respiration to generate ATP (usable cell energy), and excess glucose is stored as glycogen in the liver or cellulose in plants.

The aim of this experiment is to determine the optimal temperature for diastase activity. Solutions of diastase and starch will be heated at different temperatures, and then iodine solution will be added to determine the concentration of starch remaining. Iodine solution turns a deep blue-black colour in the presence of starch, but will remain gold if it cannot detect any. It is expected that the enzyme-substrate solution at 40°C will be the most golden solution, as it the closest to the optimal temperature of the enzyme in the body.

Methodology:

Initially, a control was established by adding 4ml of starch solution to a test tube, and then 1 drop of iodine solution. The colour was then noted.

The equipment was set up, and 5 test tubes were numbered. 4ml of 1% starch solution and 1ml of 1% diastase solution was placed into each tube using graduated measuring cylinders as shown in Figure 5, and then each was placed into a water baths at either 0, room temperature, 40, 60, or 80°C. The solutions were left for 15 minutes, then 3 drops of 1.0M HCl solution was added to stop the reaction. The tubes were swirled to mix the solutions, then removed from the water bath. 1 drop of iodine solution was added to each tube, and mixed. The colour of the solution was then compared to the control and recorded.

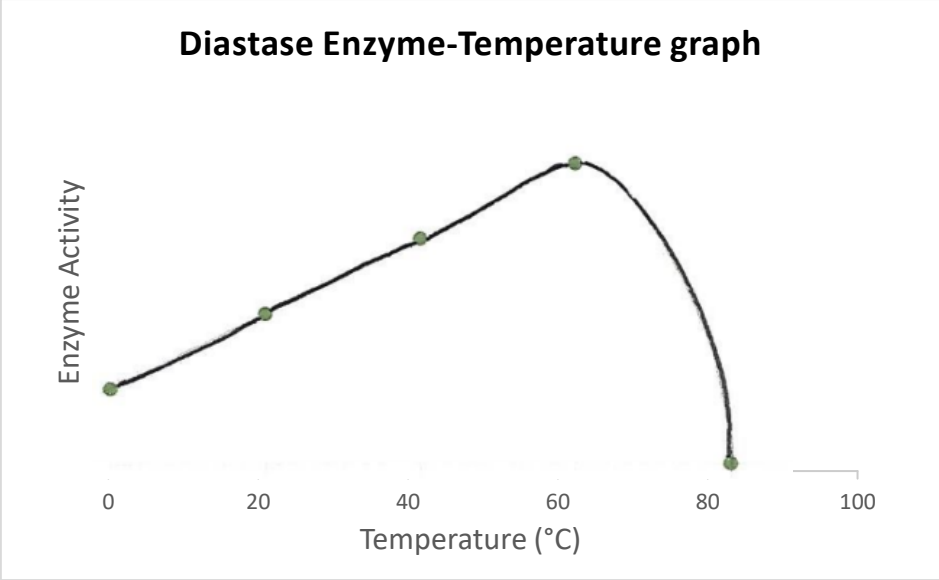
To ensure the results were reliable, the experiment was replicated 6 times to ensure no outliers were included in the data averages. Only the independent variable, temperature, was changed between experiments to ensure it was the sole factor affecting the change in results. Variables such as the amount of each solution in the test tube and time in the water bath were controlled, and cross contamination was avoided by using different equipment with different solutions. The diastase and starch solutions were also kept in the fridge to maintain a constant temperature between experiments.

Both HCl and iodine solution are corrosive if they come into contact with skin or are inhaled (Ward, 2016), so gloves and safety goggles were worn to protect organs against possible contact and the solutions were kept away from the face. Care was also taken with the water baths to ensure the skin was not burned.

Results:

Colour Observations	0°C	Room temp (19°C for tests 1-3, 20°C for test 4-7)	40°C	60°C	80°C
Run 1	Dark pink/brown	Medium pink/brown	Light pink/brown	Clear	Medium-dark blue
Run 2	Dark pink/brown	Medium pink/brown	Light-medium pink/brown	Brown	Very dark blue/black
Run 3	Pink/brown	-	Yellow/ brown	Brown	Black
Run 4	Pink	Pink/ brown	Brown	Gold/brown	Black
Run 5	Pink	Pink	Brown	Gold/brown	Purple/ black
Run 6	Pink	Pink/brown	Light brown	Gold	Black
Run 7	Pink	Brown	Brown/yellow	Gold	Dark black
Average colour	Pink	Pink/brown	Brown	Gold	Black

Iodine test for starch	Amount of starch remaining	Enzyme activity level
Dark blue-black	All	None (0)
Blue	Most	Low (1)
Light brown	Some	Moderate (2)
Gold	None	High (3)



Discussion:

From the results in Table 1, the average colour result at each temperature shows the 60°C solutions turned gold, the 80°C solution turned black, while the others ranged from pink (0°C) to brown (40°C) after iodine solution was added. Relating the data to the information in Figure 3, this demonstrate that the 60°C solution had little starch present after 15 minutes, the 0-40°C solutions had some original starch left, while the 80°C had all original starch present.

The data can be used to create an enzyme-temperature graph. As diastase breaks down starch, the results demonstrate that diastase was most active at 60°C, indicating it was the optimal temperature with the highest rate of substrate to product conversion. As the temperature decreased, the particles experienced less kinetic energy and therefore the reaction rate decreased, demonstrated by a downward slope from 60 to 0°C. However, 80°C showed no in change in starch levels, indicating the temperature denatured the enzyme. While these results do not support the hypothesis, they do support known chemical analyses of diastase (Enzyme Education Institute, 2018). The difference between the hypothesis and results could be due to the fact that diastase is more commonly found in foods rather than the human body which the hypothesis was based on. Many of the foods that contain diastase are subjected to high temperatures to break down the starch, which the optimal temperature supports.

One error that occurred during the first test was the 60°C solution turning clear. The exact cause is not known, but water may have potentially mixed with the solution in the water bath. Consequently, this colour observation was used an outlier so did not influence final results. Another error that occurred in run three was that a warm, non-homologous starch solution was used, meaning diastase activity was increased before the experiment started and more change was observed in the final solutions. The room temperature solution was also accidentally tipped out, meaning the average was calculated from less runs and could be less reliable.

This experiment could be extended by using smaller temperature increments closer to 60°C to determine a more precise optimum temperature, and by analysing results quantitatively through the use of a UV-Visible Spectrophotometer or Turbidity machine for more reliable and precise results.

Conclusion:

In this experiment, the optimal temperature of the enzyme diastase was determined by comparing its conversion of starch to glucose. The results showed that 60°C was the optimal temperature for substrate to product conversion, and while the enzyme worked at temperatures lower than this, it was denatured and did not work above 60°C.

Bibliography:

Enzyme Education Institute, (2018). *Enzyme Technical Data Sheet- Diastase* [online]. Available at: https://enzymeeducationinstitute.com/enzymes/diastase/ [Accessed 27 Sep. 2018]

IGCSE Biology, (2016). *Graphs for change in Enzyme Activity* [online]. Available at: http://igbio.tumblr.com/post/140640684431/graphs-for-changes-in-enzyme-activity [Accessed 15 Sep. 2018]

Jones, S., Borger, P., Duncan, J., Chiovitti, T. (2016*). Nelson Biology VCE Units 3 & 4.* 3rd ed. Melbourne: Cengage Learning Australia

Khan Academy, (2018). *Enzymes and the Active Site* [online]. Available at: https://www.khanacademy.org/science/biology/energy-and-enzymes/introduction-to-enzymes/a/enzymes-and-the-active-site [Accessed 15 Sep. 2018].

MSDS online, (2014). *Hydrochloric Acid Hazards and Safety Tips* [online]. Available at: https://www.msdsonline.com/2014/09/10/hydrochloric-acid-hazards-safety-tips/ [Accessed 6 Sep. 2018]

Orphardt, O (2003). *Virtual Chembook (Starch- Iodine)* [online]. Available at: http://chemistry.elmhurst.edu/vchembook/548starchiodine.html [Accessed 13 Sep. 2018]

Ward, V. (2016) *Chemical Management Handbook for Australian schools*. Australia: Australian School Science Information for teachers and technicians