Biology Practical Report

Enzyme Reactions

Aim

To investigate the effect of substrate concentration, pH levels and temperature on the rate of enzyme reaction.

Hypothesis

With certain levels of pH and substrate concentration and temperature, there will be an optimum level of enzyme activity. This is to say that when all the conditions are at the correct level, the enzyme reaction will be optimal. This will most likely echo the levels found in living organisms.

Method

Part 1 – Substrate concentration

The variables for this component of the experiment are (dependant) the enzyme activity and (independent) the substrate concentration.

1. Grind 10mg of liver using a mortar and pestle, adding 5ml of distilled water. This will allow you to obtain a "slurry" of liver enzyme.
2. With some medium grade filter paper, cut 10 small squares of approximately $5mm^2$.
3. Into FOUR separate test tubes, place 10 drops of detergent.
4. Label the test tubes "control", "3%", "6%" and "9%".
5. Place the squares of filter paper into the slurry.
6. Remove one square, and place it into the "control".
7. Record the froth level after 30 seconds. DO NOT STIR OR SHAKE THE TEST TUBE.
8. Remove one square, and place it into the "3%".
9. Into the "3%" test tube, place 5mL of 3% Hydrogen Peroxide (H_2O_2).
10. Record the froth level after 30 seconds. Do not stir or shake the test tube.
11. Remove one square, and place it into the "6%".
12. Into the "6%" test tube, place 5mL of 6% Hydrogen Peroxide (H_2O_2).
13. Record the froth level after 30 seconds. Do not stir or shake the test tube.
14. Remove one square, and place it into the "9%".
15. Into the "9%" test tube, place 5mL of 9% Hydrogen Peroxide (H_2O_2).

16. Record the froth level after 30 seconds. Do not stir or shake the test tube.
17. Repeat steps 8-15 three times, using new test tubes and new squares of filter paper.
18. Collate the results for each level of substrate concentration. Record the average.
19. Determine the most effective level of substrate concentration.

Part 2 – Temperature

The variable for this component are (dependant) the substrate concentration, 6%, enzyme activity and (independent) temperature.

1. Grind 10mg of liver using a mortar and pestle, adding 5ml of distilled water. This will allow you to obtain a "slurry" of liver enzyme.
2. With some medium grade filter paper, cut 24 small squares of approximately $5mm^2$.
3. Into TWELVE separate test tubes, place 10 drops of detergent.
4. Label two test tubes as follows; "0-1", "35", "40", "60", "80", "100", "0-1~control", "35~control", "40~control", "60~control", "80~control", "100~control"
5. Prepare a water bath for each of the different temperatures.
6. Place the two corresponding test tubes into their designated water baths.
7. Into the "control" test tubes, place a single square of the enzyme-saturated filter paper
8. Place these test tubes into there respective water baths.
9. Record the froth level after 30 seconds. Do not stir or shake the test tubes.
10. Repeat step 7 and 8 using the other test tubes.
11. Record the froth level after 30 seconds. Do not stir or shake the test tubes.

Part 3 – pH levels.

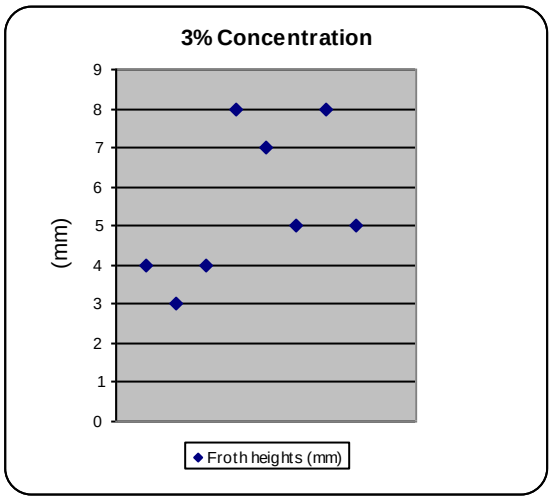
The variable components for this experiment are (dependant) the substrate concentration, 6%, the temperature, 35°C-40°C, and the enzyme activity. The independent variable for this part of the experiment is the level of pH concentration.

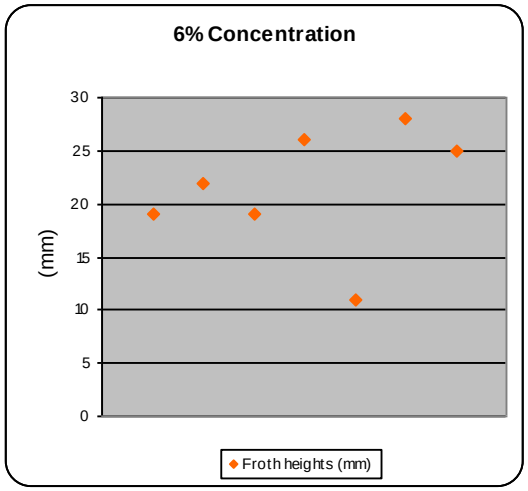
1. Grind 10mg of liver using a mortar and pestle, adding 5ml of distilled water. This will allow you to obtain a "slurry" of liver enzyme.
2. With some medium grade filter paper, cut 10 small squares of approximately $5mm^2$.
3. Into SIX separate test tubes, place 10 drops of detergent.

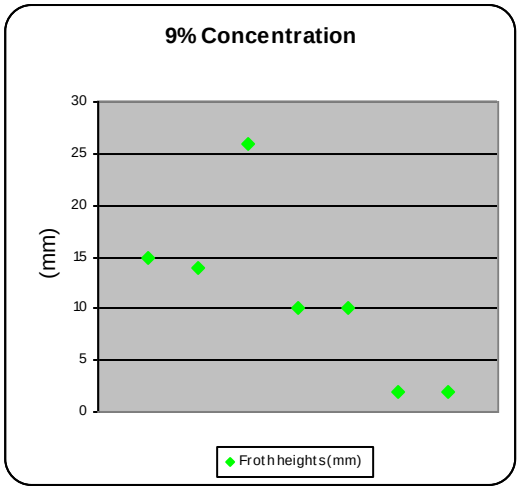
4. Label the test tubes “control”, “3”, “7” and “9-10”.
5. Place a single square of the enzyme-saturated filter paper into each of the test tubes
6. Place the pH 3, pH 7 and pH 9-10 into their respective test tubes.
7. place 10ml of 6% Hydrogen Peroxide (H_2O_2) into each of the test tubes
8. Record the froth heights after 30 seconds. <u>Do not stir or shake the test tubes.</u>

Results

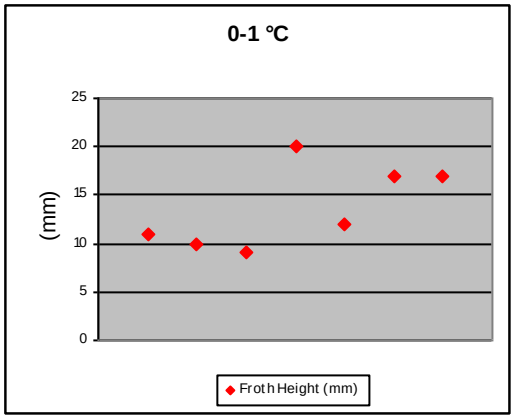
Substrate Concentration	Froth heights (mm)	Control (mm)	Average
3%	4,3,4,8,7,5,8,5	0	5.375
6%	19,22,19,26,11,28,25	0	21.43
9%	15,14,26,10,10,2,2	0	11.28

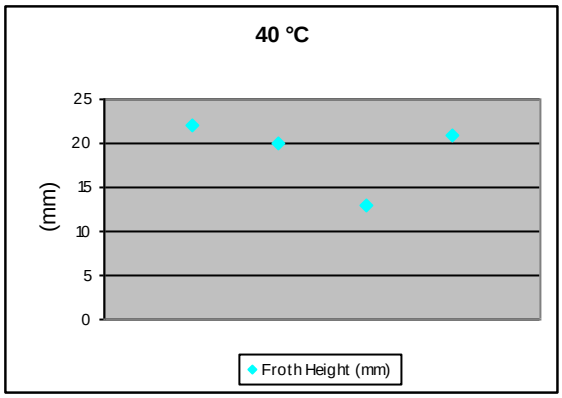


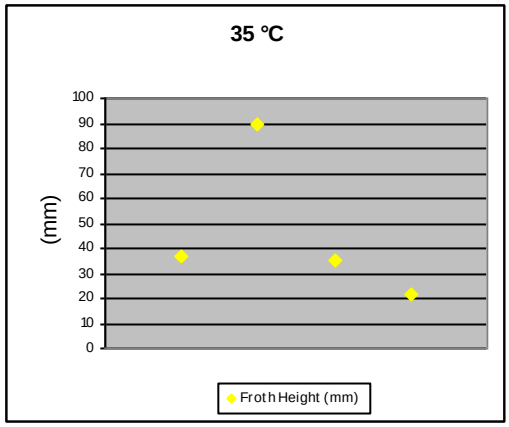




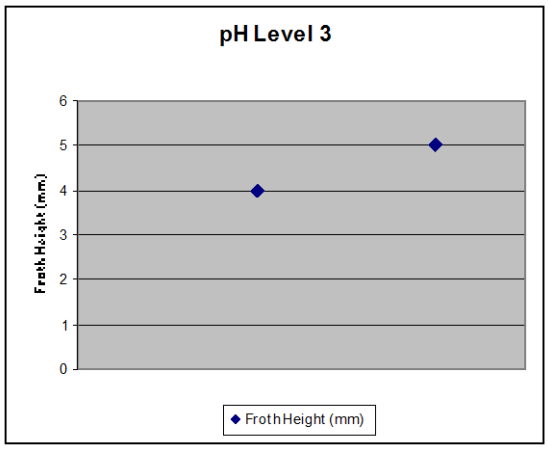
Temperature (°C)	Froth Height (mm)	Control (mm)	Average
0-1	11,10,9,20,12,17,17	0	13.71
35	37,90,35,22	0	54.75
40	22,20,19,21	1	20.5
60	20,15,22	0	19
80	2,10,10,1	0	5.75
100	2,2,3	0	2.33

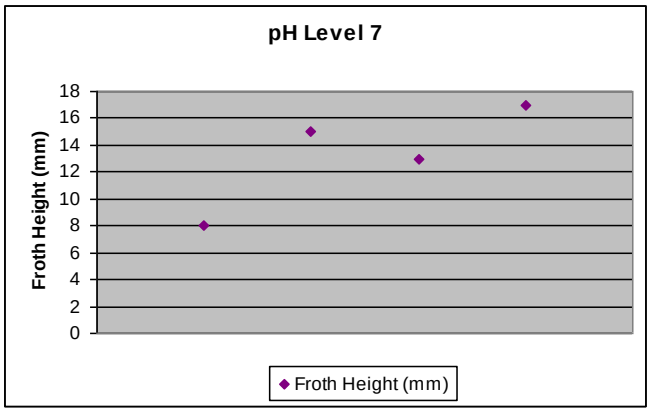


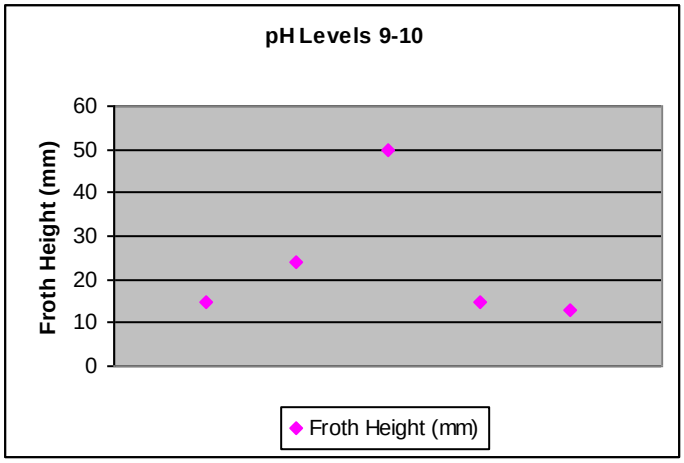




pH Level	Froth Height (mm)	Control	Average
3	4,5	0	4.5
7	8, 15,13,17	0	13.25
9-10.0	15,24,50,15, 17	0	23.4







Overall, each condition and each of the tested independent variables yielded similar results each time that specific experiment was carried out. As with all experiments there were trends among the results. These trends depict what would appear to be the ideal conditions for the most enzyme reactivity. The occasional “odd” result is most likely due to an error made by a student; these are addressed in detail in the discussion. The results from these experiments have shown that the catalyse enzyme reacts most effectively under specific conditions. These conditions are with 6% substrate concentration, approximately 35°C and with a pH level of approximately 9-10.

Discussion

This experiment was carried out with as much attention to details as was possible in a high school laboratory. Many precautions were taken to minimise error margins. These measures included using the same sample of liver for all experiments, performing the experiment at the same temperature, using the same apparatus for the repeated steps of the experiments, having the same person record the results, add substrate and enzyme etc. and the amount of water used to obtain a slurry of catalyse. However there were still some results that were indicative of error.

The errors in this experiment did not necessarily result in a failed experiment; moreover they led to results that were substantially apart from the other results. Some examples of these errors are the 2mm froth height for the 6% substrate concentration and the 90mm froth height for the 35°C experiment. The causes for these "errors" could be due to a number of factors. There may have been more substrate added to those particular test tube, which would have surrounded the enzyme not allowing for any other substrate molecules to react with it, or the filter paper square may have been slightly larger, or of a finer grade, enabling it to absorb more of the catalyse, thus the enzymes would have a higher surface area leading to a more intensified reaction.

Another point of interest regarding the pH results is the lack of repeat experiments for the pH 3 test. Having only obtained 2 results from this, we cannot be sure of what the enzymes' reactions are. Also the 50mm result in the pH 9-10 experiment is particularly interesting. This result may be high due to a student shaking or bumping the test tube whilst the reaction was taking place or during the recording period. By bumping the test tube, the enzyme is disturbed and the substrate is dislodged. This allows for new reactions and, subsequently, a higher froth.

From the results it would seem that enzymes work best under certain conditions or in a specific environment. This is supported on the HSC Online website, which states; *"Enzymes work optimally in an environment where their optimum temperature and pH conditions are met. At temperatures and pH values other than the optimum, the enzymes fail to work as efficiently as they should or not at all."*

Ideally this experiment would have been carried out on one day. Such variables like room temperature, equipment and the amount of substrate used may have changed. Different pipettes may dispense differing quantities of liquid, so more substrate or detergent may have been entered into the test tubes. Another improvement in the experiment would be to reduce the number of people involved. With less people, there is a lesser chance of error and fewer discrepancies. In addition, having each of the groups carry out the entire experiment, rather than just the 3% substrate or just the pH 9-10

experiments, would allow for a wider range of results and a more accurate reading of the ideal conditions for enzyme reactivity.

Conclusion

Therefore, it can be concluded that enzymes will react more efficiently within a narrow band of conditions. These conditions are:

- With 6% substrate concentration, this experiment used Hydrogen Peroxide (H_2O_2) as the substrate;
- At an approximate temperature of 35°C-40°C for the surrounding environment, this experiment used a water bath to obtain this; and
- A pH reading of 9-10.

As these conditions provided the most convincing results (highest froth heights), one can deduce that in the natural environment, enzymes work under similar if not identical conditions. Or if this is not the case, then it would seem that certain enzymes will work most efficiently under specific conditions.

References

The results for both the substrate concentration and temperature were from first hand investigation. The pH results were obtained from another student.
The website mentioned in the discussion can be found at
http://hsc.csu.edu.au/biology/core/balance/9_2_1/921net.html#net3