



**Investigate The Effect of
Temperature On The Rate
Of Catalase Activity
Biology – Leaving Cert
Experiments**

Materials/Equipment

Enzyme source eg. Radishes

Knife

Hydrogen peroxide (20% or less)

Chopping board

Buffer solution (pH9)

Electronic balance

Washing-up liquid

Weigh boats

Boiling tubes

Dropper

Syringe

Test-tube holder

Graduated cylinders (100cm³)

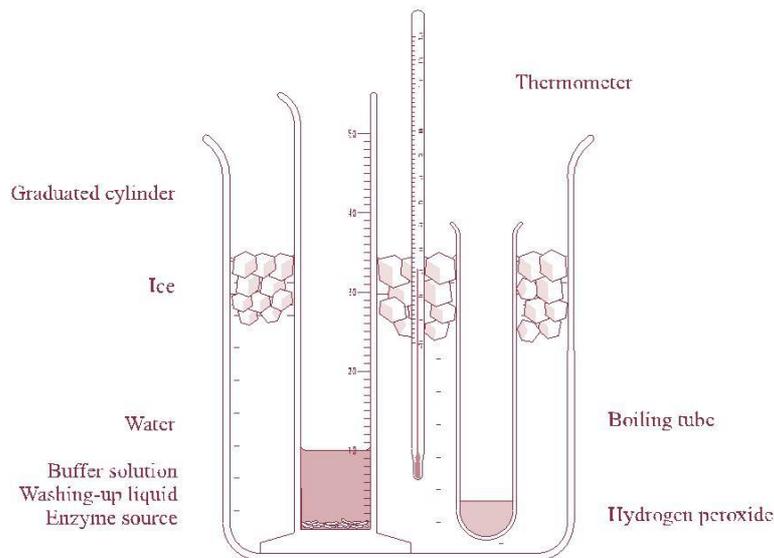
Disposable gloves

Water baths (0 °C – 60 °C)

Timer

Thermometer

Test-tube rack



Reactants cooling to 0 °C

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 20 cm³ of the buffer to the graduated cylinder.
3. Using the dropper, add one drop of washing-up liquid.
4. Add 5 g of finely chopped radish to the cylinder.
5. Add 2 cm³ of hydrogen peroxide to the boiling tube.
6. Stand the cylinder and the boiling tube in an ice-cold water bath until the desired temperature (0 °C) is reached.
7. Pour the hydrogen peroxide into the cylinder.
8. Note the volume in the cylinder immediately and record.
9. Read the volume again after a measured amount of time, e.g. 2 minutes, and record.
10. Subtract the initial volume from the final volume to get the volume of foam and record.
11. Repeat the procedure from step 3 for at least four more temperatures, to include a sample in the 50 °C – 60 °C range.
12. A graph should be drawn of enzyme activity (volume of foam after 2 minutes) against temperature. Put temperature on the horizontal axis.

Result

Temperature (°C)					
Initial volume (cm³)					
Final volume (cm³)					
Volume of foam produced (cm³)					

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF TEMPERATURE ON THE RATE OF CATALASE ACTIVITY

Following instructions

Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus

Prepare the enzyme source
Use the syringe
Use the graduated cylinder
Use the thermometer
Use the electronic balance
Set up and maintain the water baths
Use the test-tube holder
Use the timer

Observation

Observe the evolution of bubbles in the mixture
Observe the rise of foam in the cylinders

Recording

Write up the procedure
Record the volume of foam
Tabulate results
Record the temperature each time the procedure is repeated
Draw a graph with labelled axes

Interpretation

Draw reasonable conclusions from your observations and results

Application

Become aware of any other application(s) of what you learned in this activity

Organisation

Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Effect of temperature on enzymes

Each enzyme has its own optimum temperature, that is the temperature at which it works most efficiently. This optimum temperature may vary, for a given enzyme, depending on its source.

Below the optimum temperature, increasing temperature will increase the rate of the reaction. This is because temperature increases the kinetic energy of the system, effectively increasing the number of collisions between the substrate and the enzyme's active site. The effect of temperature on the rate of a chemical reaction is described by the term "temperature coefficient" (Q10).

$$Q_{10} = \frac{\text{rate of reaction at } (T + 10) \text{ } ^\circ\text{C}}{\text{rate of reaction at } T \text{ } ^\circ\text{C}}$$

T = Temperature

Many enzymes have a Q10 of between two and three which means that, provided that the temperature is not so high that it causes denaturation, an increase in temperature of 10 °C will speed up the reaction by a factor of two to three i.e. double or treble it (Q10 rule).

Catalase

All enzymes have a characteristic turnover number, which is the number of substrate molecules converted to product per second. One of the fastest known enzymes is catalase, which converts up to 10,000,000 hydrogen peroxide molecules per second into water and oxygen. The bubbles that appear in the foam are pure oxygen bubbles generated by the catalase.

For further background information refer to the investigation on the effect of pH on the rate of catalase activity.

Advance preparation

- Prepare or obtain the buffer solution.
- Buy fresh radishes.
- Set up the water baths and check the temperatures with a thermometer.

Helpful hints

- The enzyme source must be fresh.
- Besides radish there are many other good sources of catalase. Liver is the best source but celery and potato are also good. These can be used chopped, as described in the investigation, or extracts can be prepared. To prepare a celery extract, chop three stalks and macerate them in a blender in 100 cm³ of distilled water. Filter through a coffee filter. If using liver, macerate 5 g in 100 cm³ of distilled water and strain through a household sieve. Use 1 cm³ of these extracts in each cylinder. Increase this volume if activity is low.
- A more accurate value for optimum temperature may be obtained by carrying out the investigation at more than the minimum five temperatures mentioned above.
- Enzyme preparations lose activity very quickly. Therefore, the enzyme extract must be prepared immediately before use.
- If activity is high, e.g. when using liver, use larger cylinders.
- When using radish or potato as a source of catalase, lack of uniformity in the surface area of the pieces can be reduced by cutting the vegetable in slices and using a cork borer to divide each slice up into equal sized pieces. Use an equal number of pieces from each slice in each cylinder.
- The concentration of hydrogen peroxide may be expressed both as percentage and volume e.g. 30% (100 vol.).
- 6% (20 vol.) hydrogen peroxide is available in pharmacies. Other percentages are available from laboratory suppliers. Concentrations below 20% are irritants, concentrations above this are corrosive and cause burns.
- Hydrogen peroxide should be stored in a lightproof bottle because light accelerates its decomposition.
- Buffer tablets and capsules are available. Make these up with distilled water according to the instructions on the container. Liquid buffers may also be purchased.
- Higher concentrations of hydrogen peroxide than indicated in the investigation may require a greater volume of buffer to maintain pH.
- A trial run of the investigation should be carried out in advance to determine the best conditions.

