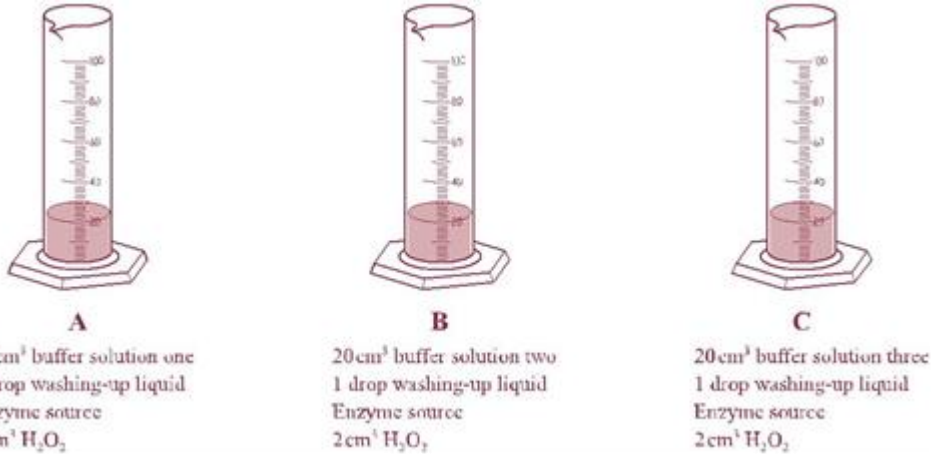
Investigate The Effect Of pH On The Rate Of Catalase Activity

Biology – Leaving Cert

Experiments

Materials/Equipment

Enzyme source eg. Radishes	Thermometer
Hydrogen peroxide (20% or less)	Knife
Range of buffer solutions – acidic, neutral and alkaline	Chopping board
pH paper	Electronic balance
Washing-up liquid	Weigh boats
Large beaker of water at 25°C	Disposable gloves
Graduated cylinders (100 cm^3)	Labels
Syringe	Timer
Boiling tubes	Dropper



Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 20 cm^3 of one of the selected buffers to a 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 cm^3 of hydrogen peroxide to a boiling tube.
6. Stand the cylinder and the boiling tube in the beaker of water at 25 °C.
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 each of the other buffer solutions.
12. A graph should be drawn of enzyme activity (volume of foam) against pH. Put pH on the horizontal axis.

Result

pH of buffer	Initial volume (cm^3)	Final volume (cm^3)	Volume of foam produced (cm^3)

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF pH 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

Maintain constant temperature (25 °C) in the beaker

Obtain enzyme extract

Use the syringe

Use the graduated cylinder

Measure pH

Use the timer

Use the electronic balance

Observation

Appreciate the use of washing-up liquid Note the evolution of bubbles in the mixture Note the rise of foam in the cylinders

Note the colours on the pH papers before and after dipping in solutions

Recording

Write up the procedure

Record the volume of foam per unit time

Tabulate the results

Record the pH in each cylinder

Draw a graph with labelled axes

Interpretation

Draw 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

In higher organisms, catalase occurs in cell organelles called peroxisomes. It is one of the fastest reacting enzymes known. It catalyses the breakdown of hydrogen peroxide to water and oxygen.

Superoxide radicals ($O2^-$) are generated during aerobic respiration when a small amount of the oxygen that normally forms water gains an electron. Excess superoxide may be converted to more damaging hydroxyl radicals. Within cells the enzyme superoxide dismutase removes superoxide by converting it to hydrogen peroxide. Hydrogen peroxide is toxic to the body and is broken down by catalase. Hydrogen peroxide is also produced by white blood cells. They produce it during phagocytosis to kill microorganisms.

Enzymes function over a narrow pH range. Each enzyme has an optimum pH and as the pH goes above or below this the activity of the enzyme drops. Extremes of pH denature enzymes.

Changes in pH alter the ionic charge of the acidic and basic groups that exist on an enzyme and on its substrate. In an enzyme these charges help to maintain the shape of its active site. Changes in pH influence the formation and decomposition of the enzyme-substrate complex and thus the activity of the enzyme.

Advance preparation

- Prepare or obtain buffer solutions.
- Obtain fresh radishes.

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 celery extract, chop 3 stalks and macerate them in a blender in 100 cm^3 of distilled water. Filter through coffee filter. If using liver, macerate 5 g in 100 cm^3 of distilled water and strain through a household sieve. Use 1 cm^3 of these extracts in each cylinder. Increase this volume if activity is low.
- 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.
- Use a large beaker as a water bath. The catalase reaction is exothermic and the large volume of water will act as a heat sink. It will also protect the bench top from spillages

and overflowing foam.

- 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.