

Ib bio experiment on effect of substrate conc on enzyme activity

[Psychology](#), [Psychotherapy](#)



The effect of substrate concentration on the rate of enzyme activity of Catalase

Aim To investigate the effect of substrate concentration (manipulated by increasing concentration of hydrogen peroxide) on the rate of enzyme activity of catalase, produced by liver cells, on the decomposition of hydrogen peroxide.

Introduction Enzymes are biological catalysts that increase the rates of reactions. In an enzyme-catalyzed reaction, the substrate binds to the active site and forms enzyme-substrate complex with the enzyme through the lock and key method (where the lock represents the enzyme and the key represents the substrate). The enzyme then breaks the bonds in the substrate. The product of the reaction then leaves the enzyme, which remains unchanged after the reaction. Without enzymes, many essential processes, such as digestion, would occur too slowly for life to continue.

Catalase is an enzyme produced by our liver cells to break down hydrogen peroxide – a common end product of metabolism, but highly toxic to tissues if accumulated in the body – into water and oxygen. The equation of the reaction is as follows: $2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}$ Catalase.

In this experiment, we obtain a 6% hydrogen peroxide solution from a pharmacy and extract equal concentrations of catalase from liver cells. Filter paper discs are dipped into the catalase solution before they are submerged in a hydrogen peroxide solution. The oxygen produced from the enzyme reaction will form on the discs and cause the disc to be buoyant enough to float upwards. We can investigate the effects of substrate concentration on the rate of reaction by catalase by using different concentrations of hydrogen

peroxide solution and measuring the rate of reaction by measuring the time taken for the disc to float to the surface when sufficient oxygen is produced.

The hypothesis for this experiment is that the rate of reaction will increase with the increase of hydrogen peroxide concentration if the other factors of enzyme activity (such as temperature, pH, and enzyme concentration) are kept constant. However, the rate of reaction will stop increasing with hydrogen peroxide concentration at a point where the enzyme concentration becomes a limiting factor. At high substrate concentrations, most of the active sites available are occupied since they are saturated with substrate molecules at any given time. Hence, a further increase in substrate concentration will not cause the rate of decomposition of hydrogen peroxide to increase. As such, the expected graph from this experiment is as follows: Expected graph of rate of reaction against the concentration of a substance.

Variables Dependent variable: Rate of enzyme activity of catalase in terms of time taken for the disk to float to the surface of the hydrogen peroxide solution when sufficient oxygen is produced. Once the filter paper disc has reached the bottom of the test tube, the stopwatch is started. The stopwatch is stopped once the disc has reached the surface of the hydrogen peroxide solution. The recorded timing indicates the amount of time taken for the disk to float to the surface of the hydrogen peroxide solution.

Independent variable:

- The concentration of hydrogen peroxide solution.
- Add different volumes of water to the different volumes of 6 % hydrogen peroxide solution.

Controlled variable(s):

1. Enzyme concentration:

- We are provided with a homogenous liquid liver solution. Hence, the concentration of catalase is constant throughout the liquid liver solution.
- The volume of hydrogen peroxide solution.
- The volume of hydrogen peroxide solution in each test tube is 5cm³.
Controlling the volume of hydrogen peroxide solutions ensures that the same amount of hydrogen peroxide molecules (substrates) is available for reaction in the test tube.

2. Size of test tubes. The test tubes used each time must be of the same size, length, and volume. This is to ensure that the distance the filter paper disc has to travel (between the bottom of the test tube to the surface) is the same for each time.

3. Filter paper disc The filter paper discs should be of the same diameter and of the same thickness. This is to ensure that the same amount of oxygen gas is required to lift it to the surface each time thereby enabling us to determine the time taken for it to do so.

4. Temperature:

- Enzyme activity is affected by temperature. The experiment is carried out at room temperature (25 °C), which is assumed to remain constant throughout the duration of the experiment.
- Digital stopwatch, accurate to 0.01 s.

- 6 Test tubes (Same size and length).
- 6 Test tube holders.
- Wooden stick.
- 500 cm³ beaker 6 measuring cylinders Materials.
- 6 % hydrogen peroxide solution provided by the teacher.
- Homogenous liquid liver solution provided by the teacher.
- 30 Filter paper discs provided by the teacher.
- Distilled water Procedure 1. 0. 75 % hydrogen peroxide solution is prepared by measuring 3. 00 cm³ of 6 % hydrogen peroxide using a measuring cylinder and then diluting it with 21. 00 cm³ of distilled water. 1. 50 %, 3. 00 %, 4. 50 % and 6. 00 % hydrogen peroxide solutions are prepared using the same method with corresponding volumes of 6 % hydrogen peroxide and water as shown in the table below.

The prepared hydrogen peroxide solutions are poured into test tubes. Each test tube should contain 5 cm³ of the solution. The test tubes with their solutions are placed in the test tube holder, labeled with the concentration of hydrogen peroxide solution that they contain.

1. Obtain homogenous liver liquid from the teacher and add 8 cm³ of liver liquid into each petri dish to be used in the experiment. (5 Petri dishes)
2. A filter paper disc is soaked with liver liquid in each petri dish.

3. A filter paper disc is removed from the petri dish and pushed to the bottom of a test tube with 0.75 % hydrogen peroxide solution using a wooden stick.
4. The stopwatch is started immediately when the filter paper disc touches the bottom of the test tube. The stopwatch is stopped once the filter paper disc reaches the surface. The time taken for the filter paper disc to float to the surface is recorded.
5. Steps 3 – 6 are repeated a further 5 times, using other new 0.75 % hydrogen peroxide solutions.
6. The average of the 6 readings for each hydrogen peroxide concentration is calculated and recorded. The rate of reaction is calculated by the following formula: $\text{Rate of reaction} = 1/\text{Average time}$ taken for filter paper disc to reach the surface of the hydrogen peroxide solution from the bottom.