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Tittle: Investigating the effect of pH on Enzyme

ABSTRACT

This experiment investigates how pH affects the activity of the enzyme catalase (from potato or liver) on the substrate hydrogen peroxide (H_2O_2). Reaction rate is measured as the volume of oxygen produced (or height of foam) over a fixed time at different pH values. It is expected that catalase will show maximum activity at a near-neutral pH and reduced activity in strongly acidic or basic conditions. Results will identify the enzyme's optimum pH and show how extremes of pH denature the enzyme and reduce activity.

Introduction

Enzymes are biological catalysts that speed up biochemical reactions without being consumed. Each enzyme has a three-dimensional structure with an active site; this structure is sensitive to environmental conditions such as temperature and pH. pH affects the ionization state of amino acid side chains essential for substrate binding and catalysis. Catalase decomposes hydrogen peroxide into water and oxygen:

Statement of the problem

How does the pH of the reaction medium affect the activity (rate) of catalase from potato (or liver) when decomposing hydrogen peroxide?

Hypothesis

If catalase activity depends on pH, then it will exhibit a maximum rate near its optimal pH (likely around pH 7 for plant/animal catalase), while at lower (acidic) or higher (basic) pH values its activity will decrease due to changes in enzyme structure and active site ionization.

Design of the study

Materials

Fresh potato (or fresh liver) — source of catalase (provide equal mass pieces)

3% hydrogen peroxide (H_2O_2) solution (or specified concentration)

Buffer solutions at pH: 3.0, 5.0, 7.0, 9.0, 11.0 (or pH range you prefer)

Distilled water

Graduated syringe or gas syringe / measuring cylinder (for measuring O_2 volume) OR test tubes + ruler for foam height method

Stop watch / timer

Beakers / test tubes (labelled)

Blender / mortar & pestle (to homogenize potato)

Filter paper / cheesecloth (to obtain extract)

Pipettes or dropper

pH paper / meter (to check buffer pH)

Ice bath (optional, to keep enzyme temperature constant)

Safety goggles, gloves

Variables

Independent variable: pH of reaction medium (pH 3, 5, 7, 9, 11)

Dependent variable: Reaction rate (e.g., volume of O_2 produced in mL per minute OR foam height in mm per minute)

Controlled variables: Temperature, enzyme source and amount, substrate concentration (H_2O_2), reaction volume, reaction time, mixing method

Controls

Negative control: buffer + H_2O_2 without enzyme extract (to check non-enzymatic decomposition)

Positive/control pH (optional): run at pH 7 as a standard reference

Replicates

At least 3 replicates per pH to allow averaging and basic statistics.

Materials Required

1. Source of Enzyme

Fresh potato (or liver) – cut into small pieces

2. Substrate

Hydrogen peroxide solution (3%)

3. pH Solutions

Buffer solutions at different pH (e.g., pH 3, 5, 7, 9, 11)

Distilled water

4. Apparatus & Glassware

Test tubes (15–20, medium size)

Test tube rack

Beakers (100 mL, 250 mL)

Graduated cylinder (50 mL or 100 mL)

Conical flask (optional, for mixing)

Measuring pipettes / droppers

Mortar & pestle or blender (for preparing enzyme extract)

Filter paper / muslin cloth / cheesecloth (for filtering enzyme extract)

Funnel

5. For Measurement

Stopwatch / timer

Procedure (step-by-step)

Use the same procedure for each pH; only the buffer solution changes.

1. Prepare enzyme extract (potato catalase):

Chop ~50 g fresh potato into small pieces.

Blend with ~100 mL cold distilled water for 30 s.

Filter through cheesecloth to obtain a clear extract. Keep extract on ice (or refrigerate) and use quickly.

2. Label test tubes for each pH (and replicates): e.g., pH3-1, pH3-2, pH3-3, pH5-1, … pH11-3, plus controls.

3. Prepare reaction mixtures (per test tube):

Add 5 mL of the buffer at the desired pH into the test tube.

Add a fixed volume (e.g., 1.0 mL) of H_2O_2 solution to each tube.

Equilibrate all tubes to the same temperature (e.g., room temp ~25°C) for 5 min.