(Substitute this modified procedure for the material found on pages 4-6 of the Enzymes module of SourceBook.)

Concept/Skills Development

LABORATORY ACTIVITY: STUDENT VERSION

Activity 1: The Effect of Temperature on the Enzyme Catalase

Introduction

Enzymes are protein molecules with a specific function. They serve as catalysts for biochemical reactions. Catalase is an enzyme found in fairly high concentration in several fruits and vegetables and also in baker's yeast. Its specific action is to decompose hydrogen peroxide into water and oxygen gas. This enzyme is also found in animal blood and accounts for the "fizzing" when hydrogen peroxide is placed on an open wound. The activity of enzymes is affected by a change in temperature and may be affected by the presence of various chemical species such as metal ions.

Investigation

How does temperature affect the activity of the enzyme catalase?

Safety

1. Wear protective goggles throughout the laboratory activity.

2. Observe general safety rules when conducting this activity. None of the materials or solutions pose unusual safety hazards or require special handling.

PROCEDURE

The chemical equation for the reaction we are studying is:

$$2\ H_2O_2(l) \xrightarrow{\text{catalase}} O_2(g) + 2\ H_2O(l)$$

We could collect the oxygen gas formed and measure it exactly (Procedure B). A much simpler but slightly less accurate approach is to generate the oxygen in a graduated cylinder, letting it push up a "blanket" of tiny oxygen bubbles as it is generated (Procedure A). The volume of this foam, which can be measured in the graduated cylinder, is a rough measure of the amount of gas evolved. We shall use the simpler method (Procedure A).

Procedure A: Measuring the Volume of Foam Produced by the Action of Catalase on Hydrogen Peroxide

Materials Needed

- 1 Dropping bottle of a solution of baker's yeast
- 1 Dropping bottle of Ivory clear dishwashing solution
- Distilled water in a container, along with a medicine dropper to dispense the water
- 1 Small bottle of 3% hydrogen peroxide
- 1 100-mL Graduated cylinder
- 1 10-mL Graduated cylinder
- 2 16-Ounce Styrofoam cups
- thermometer
- stirring rod
- Safety glasses
- Graph paper
- Stopwatch (preferably) or access to a watch or clock with a second hand
- Several paper towels
- 1 container for ice water
- 1 container for hot water

General Instructions

This experiment is to be done by a group of three people. Person A mixes the chemicals, reads the foam volume, and cleans up. Person B monitors the time, requests foam volume readings at specific times, and records data. Person C constructs the graphs and maintains the temperature of the constant temperature bath.

Part 1-A -- Measuring The Progress Of The Reaction At Room Temperature (25°C)

This experiment is conducted in an insulated water bath. The bath is simply two nested Styrofoam cups with water in the inner cup. The temperature of the water must be monitored frequently by use of a thermometer. DO THIS CAREFULLY TO AVOID BREAKING THE THERMOMETER. If the temperature of the water is above 25°C, add a small amount of ice water (not ice) with stirring to lower the temperature to exactly 25°C. If the temperature of the water is below 25°C, add a small amount of hot water with stirring to raise the temperature to exactly 25°C.

1. Thoroughly shake the bottle containing the baker's yeast mixture. Then put 18 drops of the mixture into a 100-mL graduated cylinder.
2. Add 19 drops of distilled water.
3. Add 3 drops of Ivory Dishwashing Liquid (clear).
4. Detach the cylinder from the base of the 2-part, 100-mL graduated cylinder. Swirl it gently to mix the contents, and then put the cylinder into the Styrofoam bath containing water at 25°C.* The temperature must be monitored on a continuing basis. Use ice water or hot water as needed to maintain the temperature.
5. Pour 8.00 mL of 3% hydrogen peroxide into a 2-part, 10-mL graduated cylinder.
6. Detach the 10-mL cylinder containing the hydrogen peroxide from its base and put it into the water bath already holding the 100-mL graduated cylinder.*

---

* The water bath temperature is most likely to change at these points after a graduated cylinder has just been put into the bath.

7. Allow the two graduated cylinders to remain in the water bath for 5 minutes so their contents can reach the temperature of the water bath.*

8. Tilt the 100-mL graduated cylinder about 20° from vertical and pour in 8 mL of 3% hydrogen peroxide, letting it run down the side of the cylinder to prevent splashing. Return the cylinder to a vertical position in the water bath. You may have to hold it to keep it vertical.

9. Record the exact time that the solutions are mixed.

10. Every 20 seconds measure the volume of the foam. Record the time and the foam volume on Data Sheet 1-A until six minutes has passed (from the start of the reaction)or until there are two consecutive identical foam volume values. At first you may need to raise the graduated cylinder from the water bath in order to read the volume.

    [Three or four seconds before the reading time the data recorder (Person B) says, "Get ready" and at the reading time says "Now." This guides Person A who is closely watching the foam volume. Person A then reads the foam volume aloud to Person B who records it on Data Sheet 1-A.]

11. Empty the contents of the 100-mL graduated cylinder into the sink. Thoroughly rinse the cylinder with running water, then one rinse with a small amount of distilled water.

12. Clean up.

Part 1-B -- Repeating the Experiment of Part 1-A

Repeat the experiment you just completed. Strive for maximum accuracy. Record your data from this repetitive experiment on Data Sheet 1-B.

Label the y-axis of a sheet of graph paper "Foam Volume in mL". Label the x-axis "Time in Seconds". Plot the data from Data Sheet 1-B on the graph paper and draw a smooth curve through the points. Label this graph "Graph 1".

Part 2-A -- Effect of Temperature on the Rate of the Reaction

In this part of the experiment you will carry out the same reaction but at some constant temperature other than room temperature. Your teacher will tell your group what temperature to use.

Maintain the specified temperature by adding either hot water or ice water with stirring to the water bath as needed.

Follow the general directions given in Part 1-A. However, keep in mind that the temperature of the reaction is no longer 25°, so a different temperature will have to be maintained.

Record your data on Data Sheet 2-A.

Part 2-B -- Repeating the Experiment of Part 2-A

Repeat the experiment you just completed. Strive for maximum accuracy. Record your data from this repetitive experiment on Data Sheet 2-B.

Label the y-axis of a sheet of graph paper "Foam Volume in mL". Label the x-axis "Time in Seconds". Plot the data from Data Sheet 2-B on the graph paper and draw a smooth curve through the points. Label this graph "Graph 2".

Data Analysis

1. According to the data you plotted on *Graph 1*, how does the volume of foam produced by the breakdown of hydrogen peroxide vary with time?

2. Your teacher will ask a member of your group to plot on a master graph your group's data for time *vs.* volume of foam at the temperature used by your group. Other groups' data for the assigned temperatures will also be plotted.

   According to data plotted on the master graph, what is the effect of temperature on the rate of enzymatic action? Account for any discrepancies from a general trend?

Implications and Applications

1. Enzymes have maximum activity at a temperature that depends upon the environment in which the organism thrives. Almost all enzymes found in the human body have maximum activity at 37°C (98.6°F). Can you explain this?
2. Most enzymes are inactivated at temperatures above 60°C. What does this tell you about the effect of heat on body functions?
3. Increasing the temperature will increase the rate of reaction. In fact, it has been estimated that increasing the temperature by 10°C will double the rate of enzymatic reactions. Do your data support this assumption?
4. Enzymes are typically found and used around the house. For example, meat tenderizer contains a *proteinase* that helps break down fiber and makes meat more tender. Would you add meat tenderizer to a steak while it is on the grill cooking?
5. Some detergents contain a proteinase enzyme to help remove grime and stains, which are largely protein. Do you get best results from these detergents in *cold* or *warm* water?
6. Just as the copper(II) ion inhibited the action of the enzyme catalase, other metal ions inhibit other specific enzymes. For example, mercury, lead, beryllium, and arsenic inhibit specific enzymes required for energy production. Why might mercury and lead ions be considered toxic?