Factors Affecting Enzyme Activity

Introduction

Hydrogen peroxide (H_2O_2) is a poisonous byproduct of aerobic metabolism. Cells of organisms that live in an oxygen-rich environment require the enzyme catalase to protect themselves from the harmful effects of hydrogen peroxide. Catalase decomposes hydrogen peroxide into oxygen and water as shown in the reaction below:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The assay system used in this lab consists of a small filter paper disc coated with the enzyme and then placed into a beaker of substrate (H_2O_2). As the enzyme decomposes the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen collect on the filter and make it rise to the surface of the hydrogen peroxide. The time it takes for the filter to rise is an indication of the rate of enzyme activity.

Rate of enzyme activity = distance (depth of hydrogen peroxide in mm)/time (in sec). We will assume that each filter is coated with the same amount of catalase (except in the investigation of the effect of enzyme concentration on enzyme activity).

We will use a solution of baker's yeast (*Saccharomyces cerevisiae)* as a source of the enzyme and we will arbitrarily set the concentration of the solution to 100 units/ml.

Materials:

catalase, hydrogen peroxide (3% and 1.0%), forceps, filter paper discs, distilled water (dH_20), ice, water baths, vials, marking pencils, stopwatch or timer

Steps common to all parts of the investigation:

1. Each group will investigate and report on two of the factors. With the class, decide which factors you will investigate before you begin.
2. Using forceps, dip a filter paper disk (use the three hole punch to create disks of equal size) into the appropriate enzyme solution, then remove it and drain it quickly on a paper towel. Always swirl the enzyme solution before dipping the disks.
3. Place the disk into the beaker of hydrogen peroxide so that it sits on the bottom. When the disk touches the substrate solution, begin the timer and record the time required for the disk to rise to the surface. Remove the disk after it reaches the surface.
4. Perform 5 trials for each factor and record all your data. Pool your results with the class.

Q1. For each factor, write a hypothesis regarding the effect of that factor on the rate of the enzyme-catalyzed reaction.

A. What is the effect of enzyme concentration on enzyme activity?

1. Set up five beakers containing 40 ml of 3% hydrogen peroxide each. Measure and record the depth of the hydrogen peroxide in each beaker.
2. Dilute the enzyme as outlined below. Remember that the enzyme stock is 100 units/mL. Make each dilution in a separate, labeled beaker.

100 units/ml = 20 ml 100 units/ml
80 units/ml = 12 ml 100 units/ml + 3 ml dH_2O
50 units/ml = 10 ml 100 units/ml + 10 ml dH_2O

20 units/ml = 3 ml 100 units/ml + 12 ml dH_2O
0 units/ml = 20 ml dH_2O

3. Measure the reaction time for each enzyme solution. Perform five trials.

B. What is the effect of substrate concentration on enzyme activity?
1. Obtain a beaker of catalase at 100 units/ml
2. Dilute the substrate (hydrogen peroxide) as described below. Each dilution should be made in a separate, labeled beaker. Measure and record the depth of the hydrogen peroxide.

3.0% H_2O_2: 40 ml 3% H_2O_2
1.5% H_2O_2: 20 ml 3% H_2O_2 + 20 ml distilled water
0.75% H_2O_2: 10 ml 3% H_2O_2 + 30 ml distilled water
0.38% H_2O_2: 5 ml 3% H_2O_2 + 35 ml distilled water
0.0% H_2O_2 : 40 ml distilled water

3. Measure the reaction time for each enzyme solution. Perform five trials.

C. What is the effect of pH on enzyme activity?
1. Obtain 1 beaker of 40 ml 1% H_2O_2. Measure and record the depth of the hydrogen peroxide.
2. Label 5 small beakers as follows: pH3, pH5, pH7, pH9, pH11 and dilute catalase into the appropriate beaker as described below:

pH 3: 5 mL catalase + 5 mL pH 3 Buffer
pH 5: 5 mL catalase + 5 mL pH 5 Buffer
pH 7: 5 mL catalase + 5 mL pH 7 Buffer
pH 9: 5 mL catalase + 5 mL pH 9 Buffer
pH 11: 5 mL catalase + 5 mL pH 11 Buffer

3. Measure the reaction time for each enzyme solution. Perform five trials.

D. What is the effect of temperature on enzyme activity?
1. Set up an ice bath (0^oC), a room temp water bath, a 37^oC bath and a boiling water bath.
2. Place 5 ml of catalase at 100 units/ml in each of 4 test tubes.
3. Place 1 test tube in each of the water baths.
4. Place 40 ml 1% H_2O_2 in each of 4 beakers. Measure and record the depth of the H_2O_2.
5. Allow the catalase to incubate at each temperature for 5 minutes, then test the reaction time at each temperature using a new tube of catalase for each temperature.
6. Measure the reaction time for each enzyme solution. Perform five trials.

Questions
1. Why was it important to remove the filter paper disk as soon as it reached the surface?
2. Graph the pooled data.
3. For each factor, explain the relationship between the factor and the reaction rate.
4. Why do you think homeostasis is so important for cells and organisms?