Factors Affecting Enzyme Activity

Introduction

Hydrogen peroxide (H_2O_2) is a poisonous byproduct of metabolism that can damage cells if it is not removed. Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide into water (H_2O) and oxygen gas (O_2).

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

Remember that a catalyst is a substance that lowers the activation energy required for a chemical reaction, and therefore increases the rate of the reaction without being used up in the process. Catalase is an enzyme, a biological catalyst, for which hydrogen peroxide is the substrate.

The assay system used in this lab consists of a small filter paper disc which is coated with the enzyme and then placed into a beaker of substrate (hydrogen peroxide). As the catalyst breaks down the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen collect underneath 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 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, water, ice, water baths, vials, marking pencils, stopwatch or timer

Each group will investigate and report on two of the factors.

Steps common to all parts of the investigation:

1. 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.
2. 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. (WHY?)
3. Perform 5 trials and record all your data. Calculate the average.

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 follows. Remember that the enzyme stock is 100 units/mL. Make each dilution in a separate beaker and label each:

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. Test the reaction time for the enzyme solution at 100 units/ml
4. Repeat this procedure for each of the other enzyme dilutions
5. Perform five trials. Record your results.

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. Test the reaction time for 3% H_2O_2.
4. Repeat this procedure for each of the substrate dilutions.
5. Perform five trials. Record your results.

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 directed 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. Test the reaction time at pH 3.
4. Repeat the procedure for each pH.
5. Perform five trials. Record your results.

D. What is the effect of temperature on enzyme activity?
1. Set up an ice bath (0°C), a room temp water bath, a 37°C 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.
5. Measure and record the depth of the H_2O_2.
6. Place 1 beaker in the 0°C bath and leave the other 3 beakers at room temperature. This is necessary because heat will destroy the hydrogen peroxide.
7. Allow the catalase and substrate to incubate at each temperature for about 5 minutes, then test the reaction time at each temperature. Be sure to place the catalase from each temperature in the hydrogen peroxide of the appropriate temperature.

8. Use the second room temperature beaker of hydrogen peroxide for the boiled catalase. **DO NOT BOIL HYDROGEN PEROXIDE!**
9. Time how long it takes the filter to rise at each temperature.
10. Perform five trials. Record your results.

Data and Results:
For EACH variable the class investigated, use the AVERAGE rates from the class data to construct a graph of the independent variable vs. the dependent variable. In your discussion, be sure to address the effect of each factor.