PRELIMINARY ACTIVITY FOR

Testing Catalase Activity

(O_2 Gas Sensor)

Open Inquiry Version

Many organisms can decompose hydrogen peroxide (H_2O_2) enzymatically. Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. They act as *catalysts,* substances that speed up chemical reactions without being destroyed or altered during the process. Enzymes are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second.

H_2O_2 is toxic to most living organisms. Many organisms are capable of enzymatically destroying the H_2O_2 before it can do much damage. H_2O_2 can be converted to oxygen and water, as follows:

$$2\ H_2O_2(aq) \longrightarrow 2\ H_2O + O_2(g)$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: *catalase,* found in animals and protists, and *peroxidase,* found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this Preliminary Activity, you will use catalase in yeast to catalytically decompose hydrogen peroxide. You will use an O_2 Gas Sensor to determine the rate of catalase activity by measuring oxygen gas produced as H_2O_2 is decomposed.

At the start of the reaction, there is no product, and the O_2 concentration is the same as the atmosphere. Shortly after data collection begins, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the initial rate. In this investigation, we will refer to this as the rate of catalase activity. As the peroxide is decomposed, less of it is available to react and the O_2 is produced at lower rates. When no more peroxide is left, O_2 is no longer produced. When data collection is complete, you will perform a linear fit on the resultant graph to determine catalase activity.

After completing the Preliminary Activity, you will first use reference sources to find out more about catalase, enzymes, and enzyme activity before you choose and investigate a researchable question dealing with catalase activity. Some topics to consider in your reference search are:

- catalyst
- enzyme
- catalase
- hydrogen peroxide
- collision theory
- reaction rate

PROCEDURE

1. Obtain and wear goggles.

2. Connect the O_2 Gas Sensor to the data-collection interface. Start the data-collection program.

3. Prepare to initiate the catalase catalyzed reaction.
   a. Use a utility clamp to fasten an O_2 Gas Sensor to a ring stand.
   b. Place 10.0 mL of 1.5% H_2O_2 into a clean 250 mL Nalgene bottle. Take care to minimize depositing drops on the sides of the bottle.
   c. Place a stir bar into the bottle.
   d. Position a magnetic stirrer on the base of the ring stand.

4. Initiate the enzyme catalyzed reaction and start data collection. Complete this step *quickly*.
   a. Using micropipette, add 100 µL of enzyme suspension to the contents of the Nalgene bottle.
   b. Swirl the contents of the bottle for 2–3 seconds to ensure thorough mixing.
   c. Place the bottle onto the O_2 Gas Sensor as shown in Figure 1. Gently push the bottle up onto the sensor until it stops. The sensor is designed to seal the bottle without the need for unnecessary force.
   d. Position the O_2 Gas Sensor and Nalgene bottle assembly on the magnetic stirrer.
   e. Start the magnetic stirrer, and adjust it to a medium speed.
   f. Start data collection.

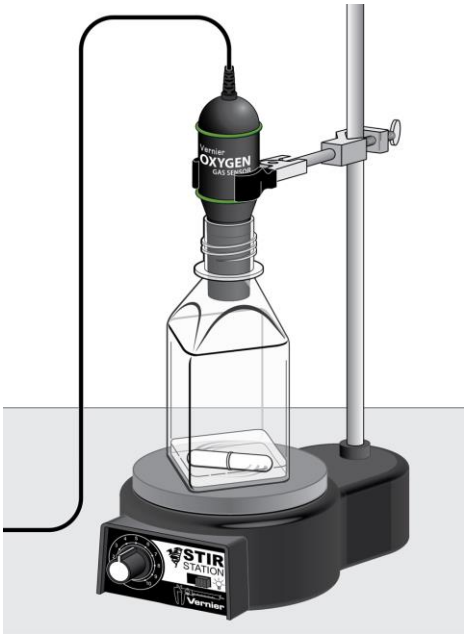

Figure 1

5. When 200 seconds have elapsed, stop data collection.

6. Remove the O_2 Gas Sensor from the Nalgene bottle. Rinse the bottle with water and dry it with a soft paper towel.

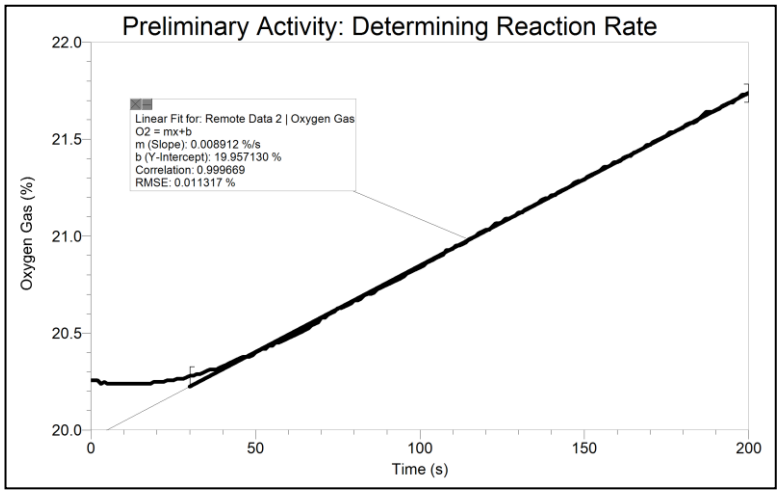

Figure 2

7. Perform a linear fit on the 30–200 s portion of the graph (see Figure 2). Record the slope of the line, *m*, as the rate of catalase activity, in % O_2/s.

QUESTIONS

1. What was the rate of catalase activity?

2. Why is it important that cells contain catalase?

3. List three factors that could possibly affect catalase activity.

4. List at least one researchable question concerning catalase activity.