**Graphical Analysis**

**Enzyme Action:   
Temperature of Denaturation**

(O2 Gas Sensor)

Enzymes are globular proteins, responsible for most of the chemical activities of living organisms. Enzymes act as *catalysts*, substances that speed up chemical reactions without being destroyed or altered during the process. They are extremely efficient and may be used over and over again. One enzyme may catalyze thousands of reactions every second. Both the temperature and the pH at which enzymes function are extremely important. Most organisms have a preferred temperature range in which they survive, and their enzymes typically function best within that temperature range. If the environment of the enzyme is too hot, acidic, or basic, the enzyme may irreversibly *denature*, or unravel, until it no longer has the shape necessary for proper functioning.

H2O2 is toxic to most living organisms. Many organisms are capable of enzymatically breaking down the H2O2 before it can do much damage. H2O2 can be converted to oxygen and water, as follows:

2 H2O2 ↔ 2 H2O + O2

Although this reaction occurs spontaneously, the enzyme catalase increases the rate considerably. Catalase is found in most living organisms.

A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions. The rate of a chemical reaction may be studied in a number of ways including

* Measuring the rate of appearance of a product
* Measuring the rate of disappearance of substrate
* Measuring the pressure of the product as it appears

In this experiment, you will measure the rate of enzyme activity after the enzyme has been exposed to various temperatures. You will use an O2 gas sensor to measure the concentration of oxygen gas formed as H2O2 is destroyed.

**Objectives**

* Measure the production of oxygen gas as hydrogen peroxide is broken down by the enzyme catalase or peroxidase after exposure to various temperatures.
* Measure and compare the initial rates of reaction for the enzyme after exposure to each temperature.

**MATERIALS**

Chromebook, computer, **or** mobile device

Graphical Analysis 4 app

Go Direct O2 Gas (GDX-O2) **or** O2 Gas Sensor (O2-BTA)\*

6 × 200 mL beakers

250 mL Nalgene® bottle

20–200 µL micropipette

200 µL micropipette tips (1 box)

1.5% H2O2

enzyme suspension

6 × 1.5 mL microtubes

Stir Station with stir bar

magnetic stir bar

utility clamp

ice

thermometers

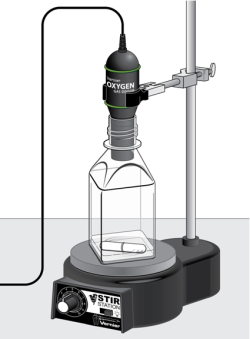
goggles

\*An interface, such as LabQuest, is required if using O2-BTA

**PROCEDURE**

**Part I  Reaction Rate at Room Temperature**

1. Obtain and wear goggles.
2. Launch Graphical Analysis. Connect the O2 gas sensor to your Chromebook, computer, or mobile device. Use an interface if necessary.
3. Create a 20–25°C water bath (see Table 1) and label the bath with the number 20 (the minimum temperature of the water bath). Use a thermometer to assist in maintaining the proper temperature for the water bath.
4. Obtain a 1.5 mL microtube. Label the microtube with the number 20 (which correlates to the minimum temperature of the water bath you will be using for this microtube). Using a micropipette, add 200 µL of enzyme suspension to the microtube. Cap the tube and then place it in the 20–25°C water bath. Let the tube sit in the water bath for at least 10 minutes. After 10 minutes have elapsed, proceed to the next step.
5. Prepare to initiate the enzyme catalyzed reaction.
   1. Use a utility clamp to fasten the O2 gas sensor to the Stir Station (see Figure 1).
   2. Place 10.0 mL of 1.5% H2O2 into a clean 250 mL Nalgene bottle. Take care to minimize depositing drops on the sides of the bottle.
   3. Place a stir bar into the bottle.



*Figure 1*

1. Initiate the enzyme catalyzed reaction and start data collection. **Note**: This step should be completed as rapidly as possible.
   1. Using a micropipette, add 100 µL of enzyme suspension from the microtube to the contents of the Nalgene bottle.
   2. Swirl the contents of the bottle for 2–3 seconds to ensure thorough mixing.
   3. Place the O2 gas sensor into the bottle as shown in Figure 1. Gently push the bottle up onto the sensor until it stops. **Note**: The sensor is designed to seal the bottle with minimal force.
   4. Position the O2 gas sensor and Nalgene bottle assembly on the Stir Station.
   5. Start the magnetic stirrer, and adjust it to a medium speed.
   6. Start data collection.
2. When 200 seconds have elapsed, stop data collection.
3. Remove the O2 gas sensor from the Nalgene bottle. Rinse the bottle with water and dry it with a soft paper towel.
4. Determine the rate of enzyme activity.
   1. Select the 30–200 s portion of the graph.
   2. Click or tap Graph Tools, , and choose Apply Curve Fit.
   3. Select Linear as the curve fit. Click or tap Apply.
   4. Record the slope, *m*, as the rate of catalase activity (in % O2/s) in Table 2. This is the reaction rate for room temperature (20–25°C).
   5. Dismiss the Curve Fit box.

**Part II  Reaction Rates after Exposure to Different Temperatures**

You will need to create a series of water baths for this part of this experiment (see Table 1). Label the water baths based on the minimum temperature of the bath (i.e., the temperature of bath 10 will be 10–15°C). Use a thermometer to assist in maintaining the proper temperature for each water bath.

|  |  |
| --- | --- |
| Table 1 | |
| Water bath temperature  (°C) | Description |
| 10–15 | 200 mL beaker filled with ice and water |
| 20–25 | 200 mL beaker filled with tap water |
| 30–35 | 200 mL beaker filled with warm water |
| 40–45 | 200 mL beaker filled with warm and hot water |
| 50–55 | 200 mL beaker filled with hot water |
| 60–65 | 200 mL beaker filled with very hot water |

1. Obtain five 1.5 mL microtubes. Label the microtubes 10, 30, 40, 50, and 60 (the minimum temperature of the water bath for each tube). Using a micropipette, add 200 µL of enzyme suspension to each microtube. Cap the tubes and then place each tube in the correlating water bath (i.e., tube 10 goes into bath 10, the 10–15°C bath). Let the tubes sit in the water bath for at least 10 minutes.
2. After 10 minutes have elapsed, place all of the tubes in the 20–25°C water bath. Wait an additional 10 minutes so each tube has time to come back to room temperature.
3. Find the rate of enzyme activity for each tube:
   1. Repeat Steps 5–9 using tube 10. Record the reaction rate in Table 1. This is the reaction rate for the enzyme after exposure to 10–15°C.
   2. Repeat Steps 5–9 using tube 30. Record the reaction rate in Table 1. This is the reaction rate for the enzyme after exposure to 30–35°C.
   3. Repeat Steps 5–9 using tube 40. Record the reaction rate in Table 1. This is the reaction rate for the enzyme after exposure to 40–45°C.
   4. Repeat Steps 5–9 using tube 50. Record the reaction rate in Table 1. This is the reaction rate for the enzyme after exposure to 50–55°C.
   5. Repeat Steps 5–9 using tube 60. Record the reaction rate in Table 1. This is the reaction rate for the enzyme after exposure to 60–65°C.

**DATA**

|  |  |
| --- | --- |
| Table 2 | |
| Exposure temperature  (°C) | Reaction rate  (% O2/s) |
| 10–15 |  |
| 20–25 |  |
| 30–35 |  |
| 40–45 |  |
| 50–55 |  |
| 60–65 |  |

**PROCESSING THE DATA**

Make a graph of the rate of enzyme activity *vs*. exposure temperature in Graphical Analysis or by hand. Plot the reaction rate values on the y-axis and exposure temperature on the x-axis. Use this graph to answer the questions below.

**QUESTIONS**

1. Which exposure temperature has the highest rate of enzyme activity? Which exposure temperature has the lowest? Explain.
2. How does changing the exposure temperature affect the rate of enzyme activity? Does this follow a pattern you anticipated?
3. Why might the enzyme activity decrease at higher exposure temperatures?