**Catalase Enzyme Action**

<table>
<thead>
<tr>
<th>Qty</th>
<th>Equipment and Materials</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PASPORT Xplorer GLX</td>
<td>PS-2002</td>
</tr>
<tr>
<td>1</td>
<td>PASPORT Oxygen Gas Sensor</td>
<td>PS-2126</td>
</tr>
<tr>
<td>1</td>
<td>Sampling Bottle (included with Oxygen Gas Sensor)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Balance</td>
<td>SE-8723</td>
</tr>
<tr>
<td>2</td>
<td>Beaker, 1 L</td>
<td>SE-7288</td>
</tr>
<tr>
<td>1</td>
<td>Graduated cylinder</td>
<td>SE-7289</td>
</tr>
<tr>
<td>1</td>
<td>Hot plate</td>
<td>SE-8767</td>
</tr>
<tr>
<td>1</td>
<td>Magnetic stirrer and stir bar</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Test tube</td>
<td></td>
</tr>
<tr>
<td>1 pair</td>
<td>Tongs</td>
<td></td>
</tr>
<tr>
<td>12 mL</td>
<td>Chicken liver extract</td>
<td></td>
</tr>
<tr>
<td>10 mL</td>
<td>Hydrochloric acid (HCl), 1 molar</td>
<td></td>
</tr>
<tr>
<td>100 mL</td>
<td>Hydrogen peroxide, 3%</td>
<td></td>
</tr>
<tr>
<td>500 mL</td>
<td>Ice, crushed or cube</td>
<td></td>
</tr>
<tr>
<td>1 g</td>
<td>Sodium fluoride, solid</td>
<td></td>
</tr>
<tr>
<td>10 mL</td>
<td>Sodium hydroxide (NaOH), 1 molar</td>
<td></td>
</tr>
<tr>
<td>500 mL</td>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>500 mL</td>
<td>Water, distilled</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Weighing paper</td>
<td></td>
</tr>
</tbody>
</table>

**Purpose**

The purpose of the activity is to explore some of the factors that influence the rate of enzyme activity in an organism.

**Background**

Enzymes are very important molecules found in every cell. Enzymes generally act as catalysts that increase the speed or rate at which substances in a cell get converted into other substances. Without enzymes, some reactions would take place too slowly – or might not take place at all.

Each enzyme has a different job and many enzymes must work together to keep an organism alive and healthy. In the liver, for example, there are several enzymes that act on certain toxic or poisonous compounds by removing hydrogen atoms from the poisons and transferring them to oxygen molecules. This detoxifies the poison but it creates a new compound, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) that is very active and can be harmful to the organism. Fortunately there is another enzyme in the liver that helps break down the peroxide into water and oxygen.

This enzyme is known as catalase. The catalase enzyme reduces the substrate, peroxide, to water and oxygen by the following decomposition reaction.

\[
2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2 \text{(gas)}
\]

Like all enzymes, catalase helps the reaction but does not itself get used up in the reaction. Also like other enzymes, catalase must have a proper environment in which to work. Your body’s enzymes, for example, work best when your temperature is normal (around 37° C) and when the
pH is between 7.3 to 7.4. If the environmental conditions are outside the normal range, the catalase will lose its ability to catalyze the peroxide reaction or may even be destroyed.

**Pre-lab Questions**

The catalase enzyme breaks down hydrogen peroxide and releases gaseous oxygen. Measure the gaseous oxygen level (ppm) in a solution of liver extract and hydrogen peroxide when different substances are added to the solution.

1. What effect do you think adding an inhibitor to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?
2. What effect do you think adding a base (high pH solution) to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?
3. What effect do you think adding acid (low pH) to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?
4. What effect do you think decreasing the temperature of the catalase will have on the enzymes’s ability to catalyze the breakdown of the peroxide?
5. What effect do you think boiling the catalase will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

**Safety Precautions**

- Follow all directions for using the equipment.
- Wear protective gear (e.g., safety goggles, gloves, apron).

**Procedure**

**GLX Setup**

1. Plug the end of the Oxygen Gas probe cable into the connector on the top of the PASPORT Oxygen Gas Sensor.
2. Open the GLX setup file labeled `catalase.glx` (check the appendix at the end of this activity). The file is set so the sensor will measure 2 times per second.
3. Connect the Gas Oxygen Sensor into Port 1 on the top of the Xplorer GLX.
   - The Graph Screen will automatically open with O2 Concentration (%) versus Time (s).

**Sensor Calibration (Optional)**

- See the appendix at the end of this activity.
Equipment Setup
1. Put about 500 mL of water into a beaker and put the beaker on a hot plate. Start heating the water to a boil.
2. Put a spin bar into the sampling bottle and place the bottle on the magnetic stirrer.

Record Data
- There are six parts to the data recording.

<table>
<thead>
<tr>
<th>Part</th>
<th>Description</th>
<th>Part</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Catalase + Hydrogen Peroxide</td>
<td>D</td>
<td>Catalase + Hydrogen Peroxide + Acid</td>
</tr>
<tr>
<td>B</td>
<td>Catalase + Hydrogen Peroxide + Inhibitor</td>
<td>E</td>
<td>Chilled Catalase + Hydrogen Peroxide</td>
</tr>
<tr>
<td>C</td>
<td>Catalase + Hydrogen Peroxide + Base</td>
<td>F</td>
<td>Heated Catalase + Hydrogen Peroxide</td>
</tr>
</tbody>
</table>

Part A: Catalase + Hydrogen Peroxide

Prepare the Mixture
1. Pour 15 mL of 3% hydrogen peroxide in a 100 mL graduated cylinder. Fill the cylinder to the 100-mL mark with 85 mL of distilled water.
2. Transfer the diluted peroxide solution to the sampling bottle.
3. Turn on the stirrer.
4. Add 2 mL of catalase extract to the dilute peroxide solution in the sampling bottle.
5. Insert the oxygen sensing element into the sampling bottle.

Record Data
6. Press the Start key on the GLX.
7. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.
8. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Leave the spin bar in the bottle.

Part B: Catalase + Hydrogen Peroxide + Inhibitor

Prepare the Mixture
9. Prepare 100 mL dilute hydrogen peroxide as before and put it into the sampling bottle.
10. Add 1 g of sodium fluoride to the dilute peroxide solution. Add 2 mL of catalase extract to the bottle.
11. Re-insert the oxygen sensing element into the sampling bottle.
Record Data

12. Press the Start key on the GLX.
13. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.
14. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Leave the spin bar in the bottle.

Part C: Catalase + Hydrogen Peroxide + Base

Prepare the Mixture

15. Prepare 100 mL dilute hydrogen peroxide as before and put it into the sampling bottle.
16. Add 10 mL of 1 molar sodium hydroxide (NaOH) to the dilute peroxide solution. Add 2 mL of catalase extract to the bottle.
17. Re-insert the oxygen-sensing element into the sampling bottle.

Record Data

18. Press the Start key on the GLX.
19. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.
20. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Leave the spin bar in the bottle.

Part D: Catalase + Hydrogen Peroxide + Acid

Prepare the Mixture

21. Prepare 100 mL dilute hydrogen peroxide as before and put it into the sampling bottle.
22. Add 10 mL of 1 molar hydrogen chloride (HCl) to the dilute peroxide solution. Add 2 mL of catalase extract to the bottle.
23. Re-insert the oxygen-sensing element into the sampling bottle.

Record Data

24. Press the Start key on the GLX.
25. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.
26. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Leave the spin bar in the bottle.

Part E: Chilled Catalase + Hydrogen Peroxide

Prepare the Mixture

27. Prepare 100 mL dilute hydrogen peroxide as before and put it into the sampling bottle.
28. Put 2 mL of catalase extract into a test tube. Put the test tube into a beaker and pack crushed or cube ice around the test tube. Add some water to the ice. Cool the test tube in the ice for 5 minutes.

29. Add the chilled catalase to the sampling bottle and re-insert the oxygen-sensing element into the bottle.

Record Data

30. Press the Start key on the GLX.

31. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.

32. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Leave the spin bar in the bottle.

Part F: Boiled Catalase + Hydrogen Peroxide

Prepare the Mixture

33. Prepare 100 mL dilute hydrogen peroxide as before and put it into the sampling bottle.

34. Put 2 mL of catalase extract into a test tube. Use tongs to hold the test tube in a beaker of boiling water. Heat the test tube in the boiling water for 5 minutes.

35. Add the heated catalase to the sampling bottle and re-insert the oxygen-sensing element into the bottle.

Record Data

36. Press the Start key on the GLX.

37. Record data for 5 minutes (or until the oxygen level stabilizes). Write a description of what happens in the solution.

38. Remove the oxygen sensing element from the sampling bottle. Dispose of the contents of the bottle as directed and rinse the bottle thoroughly. Be careful to remove the spin bar.

Analysis

1. Draw a sketch of one run of O2 Concentration versus Time graph as requested in the Lab Report section.

2. Use your recorded data to find the change in oxygen gas production for each run of data you recorded.

• In the Graph Screen, press F3 to open the ‘Tools’ menu. Select ‘Statistics’ and press ‘Activate’. The Statistics show the minimum and maximum values.
• To select a specific run of data in the Graphs screen, press ‘Activate’. This highlights the label on the vertical axis. Press the right-cursor key to highlight ‘Run #’ in the upper right corner of the Graph screen. Press ‘Activate’ a second time to show the ‘Run #’ menu. Use the up-down cursor keys to select the run you want. Press ‘Activate’ and the graph displays the run.

3. Calculate the catalase activity for each run and record your results.

• How do your results compare with others in your class?

Record your results in the Lab Report.

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Appendix:

To open a specific GLX file, go to the home screen (press ). In the home screen, select ‘Data Files’ and press ‘Activate’. Use the cursor keys to navigate to the file you want. Press F1 to open the file.

Data Files Icon

Optional: To calibrate the PS-2126 Oxygen Gas Sensor, see the instructions provided by the instructor.
Lab Report - Activity 05: Catalase Enzyme Activity

Pre-Lab Questions

The catalase enzyme breaks down hydrogen peroxide and releases gaseous oxygen. Measure the gaseous oxygen level (ppm) in a solution of liver extract and hydrogen peroxide when different substances are added to the solution.

1. What effect do you think adding an inhibitor to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

2. What effect do you think adding a base (high pH solution) to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

3. What effect do you think adding acid (low pH) to the hydrogen peroxide will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

4. What effect do you think decreasing the temperature of the catalase will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

5. What effect do you think boiling the catalase will have on the enzymes’s ability to catalyze the breakdown of the peroxide?

Data

Make a sketch of one run of O2 Concentration versus Time, including labels for the y- and x-axes.
Data Table

<table>
<thead>
<tr>
<th>Item</th>
<th>Part A</th>
<th>Part B Inhibitor</th>
<th>Part C High pH</th>
<th>Part D Low pH</th>
<th>Part E Chilled</th>
<th>Part F Heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting O2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ending O2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2 Difference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity (%/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Questions

1. What does the graph of the reaction between hydrogen peroxide and catalase tell you about enzyme activity?

2. Describe the effect of adding the inhibitor (sodium fluoride) to the peroxide before you add the catalase to the solution of peroxide? What explanation can you give for the results?

3. Describe the effect of adding the base (sodium hydroxide) to the solution of peroxide? What did the sodium hydroxide do to the pH of the solution in the flask? What does this tell you about the range of conditions in which catalase may be effective?

4. Describe the effect of adding the acid (hydrochloric acid) to the solution of peroxide? What did the hydrochloric acid do to the pH of the solution in the flask? What does this tell you about the range of conditions in which catalase may be effective?

5. Describe the effect of cooling the catalase before adding it to the solution of peroxide?

6. Describe the effect of heating the catalase to boiling before adding it to the solution of peroxide? How did the effect of cooling compare to the effect of boiling the catalase? How can you explain the difference between these two trials?