ENZYME ACTION

Introduction: Catalysts are substances that speed up chemical reactions. Organic catalysts are called enzymes. Catalase is an enzyme normally found in many plant and animal tissues. The biological purpose of catalase is to destroy toxic substances that may be introduced into cells. Also, some cells use catalase to destroy cellular debris or worn out organelles. In this lab, we will use a catalase solution from potatoes and determine the effect of temperature and pH on the action of this enzyme. The substrate of the enzyme will be 3% hydrogen peroxide (H$_2$O$_2$). Catalase works by the following mechanism:

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

The development of oxygen gas can be observed by the foam produced in a test tube.

Materials:

- Eye protection
- Red-skinned potatoes
- Scalpel or knife (for cutting and mashing the potato)
- H$_2$O$_2$
- Test tubes
- Hot water bath
- Ice bath
- pH paper
- Metric ruler
- Test tube rack
- Marker pen or pencil
- Test tube clamps
- acid (HCl)
- base (NaOH)
- Rocks or pebbles

CAUTION! HCl and NaOH are strong acids and bases. If you spill these chemicals on your skin, immediately wash in running water. Wear eye protection at all times. Be careful of hot water and hot plates.

Pre Lab Questions. (Need help? Use pg. 57 in the Modern Biology text)

1. What type of biological molecules are enzymes? (circle one)
   a. proteins  
   b. carbohydrates  
   c. lipids  
   d. nucleic acids

2. Describe how an enzyme works and its importance to all living organisms.

3. TRACE the diagram of enzyme action on page 57 of your textbook, below. Label the appropriate parts of the picture: catalase, H$_2$O$_2$, H$_2$O, and O$_2$.
4. **Define** the following terms as they apply to enzymes:

a) **Active site:**

b) **Substrate:**

c) **Denature:**

**Part A: Exploring Catalase Activity**

**Procedure:**
1. Label 4 test tubes 1, 2, 3, 4 and mark each at the 3cm mark with a marker pen or pencil.
2. Fill tube 1 to the 3cm mark with hydrogen peroxide, and then drop a small rock or pebble in.
3. Cut three 1cm³ cubes of potato, minus the skin.
4. Fill tube 2 to the 3cm mark with hydrogen peroxide, then drop in one potato cube.
5. Fill tube 3 to the 3cm mark with hydrogen peroxide, then mash or chop up a potato cube and add it to the tube.
6. Fill tube 4 to the 3cm mark with water, then mash or chop up a potato cube and add it to the tube.

Observe the reactions in the 4 tubes for 5 minutes. **Note** how vigorously each reaction “bubbles” (emits oxygen gas) in **Table 1**. Using a metric ruler, measure the height of the bubbles produced after 5 minutes.

**Part A: Results and Observations:**
After adding the H₂O₂, watch the clock and record activity based on oxygen bubbles (product) given off. **Rate the activity for each test tube using the following rating scale and complete Table 1 with these symbols:**

| +++++ | extremely active bubbling; foamy |
| + + | good bubbling |
| + | moderate bubbling |
| 0 | nonreactive; no bubbling |

**Table 1: Activity of Enzyme on Hydrogen Substrate Decomposition**

<table>
<thead>
<tr>
<th>tube</th>
<th>Test tube</th>
<th>First minute activity rating</th>
<th>5 minute activity rating</th>
<th>Height of bubbles (cm)</th>
<th>Scientific explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pebble in H₂O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Potato cube and H₂O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chopped potato and H₂O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chopped potato and water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Part A - Analysis and Conclusions:

1. What were the controls in this experiment? (hint: what conditions were the same for two or more of the tubes?)

2. What was the variable being tested in each tube? (list each of them)

3. Of all the test tubes, which one reacted the most violently with the hydrogen peroxide?

4. What are the reasons it reacted so vigorously? Think scientifically!

5. Which test tube(s) reacted the least? What scientific reasons can you give for this?

6. In a few sentences, summarize your findings from Table 1:

Part B: The Effect of temperature on an enzyme.

1. Label 3 test tubes 1, 2, and 3. Mark each tube to the 3 cm mark with a marking pen or pencil.
2. Place a 1cm$^3$ cube of potato into each test tube. Add 5 drops of water to each.
3. Place the test tube labeled “1” in the hot water bath for 5 minutes.
4. Place the test tube labeled “2” in the cold water bath for 5 minutes and leave the test tube labeled “3” temperature in the test tube rack, at room temperature.
5. After the 5-minute interval, add of H$_2$O$_2$ to the mark on each tube.
6. Wait five minutes and record the height of the bubbles in each tube in cm. Record in Table 2.
Part B

Data:

Table 2: Effect of Temperature on Catalase Reactions

<table>
<thead>
<tr>
<th>Test tube</th>
<th>Temperature (°C)</th>
<th>Height of Bubbles (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Room Temp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. What effect did (a) heat have on the activity of the enzyme? (b) What effect did cold have? (c) Room Temperature?

2. What were the controls in this experiment?

3. Which test tube reacted the least? What scientific reasons can you give for this?

4. In a few sentences, summarize your findings from Table 2, here:
Part C: The Effect of pH on Enzyme Activity:
1. Label 3 test tubes “1”, “2” and “3”. Mark each to the 3 cm mark with a marker pen or pencil.
2. Place a 1 cm³ of potato into each test tube.
3. Add 10 drops of HCl to the tube labeled “1”, 10 drops of NaOH to the tube labeled “2” and 10 drops of distilled water to the tube labeled “3”. Mix contents by gently swirling. Test the pH of each using a pH stick.
4. Wait 2 minutes, then add H₂O₂ to the 3 cm mark in each tube.
5. Wait 5 minutes for reaction to occur, then measure the height of the bubbles in cm. Record in Table 3.

Table 3: Effect of pH on Catalase Reactions

<table>
<thead>
<tr>
<th>pH</th>
<th>Height of Bubbles (cm)</th>
<th>Comments:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis and Conclusions:

1. What effect did (a) lowering the pH have on the activity of the enzyme? (b) Raising the pH?

2. What were the controls in this experiment?

3. Which test tube reacted the least? What scientific reasons can you give for this?

4. In a few sentences, summarize your findings from Table 3, here:
Cleanup:

1. All chemicals go down the sink drain, with LOTS of cold running water to dilute them.
2. All potato pieces go into regular trash can, NOT in the sink!
3. Wash and dry all test tubes after each experiment. When unused, leave them upside down in test tube rack.
4. Spray lab table with BLUE cleaning solution and wipe down with paper towels.
5. Wash your hands!

Hint: spell POTATO (singular) or POTATOES (plural). Never “POTATOE”!