Investigating Enzymes – Determining the Optimal pH for Enzyme Activity
Teacher’s Guide

This guide is a continuation of the Overview and Lab 1 document. Presented below are notes and comments regarding the laboratory material and preparation for the laboratory exercise.

Enzymes move electrons and protons to different locations within a molecule. These movements cause bonds to break between atoms which change the structure of molecules. Because the movement of electrons and protons is so important to enzyme activity, enzymes are very sensitive to the pH of reaction solution. pH is a scale that is used to measure the amount of protons (hydrogen ions) that are in a solution. The scale starts at 1 (very acidic) and increases to 14 (very basic/alkaline). Most enzymes are active in the pH range of 6-8, but some enzymes can function at a pH as low as 3 and as high as 10. In very acidic or very alkaline solutions, enzymes may change shape and/or the movement of electrons and protons may be slowed. In both cases, this can affect the speed of the enzyme reaction.

In this laboratory, you will see how pH affects enzyme reactions. This will be done by using five different solutions, called buffers. Each buffer has a different pH. Buffers are salts that create a specific pH when dissolved in water. By running a basic enzyme reaction with these different buffers, you can determine at which pH activity is greatest. Teacher’s Note: This laboratory allows the students to assess at which pH LDH is most active. In the true sense, they will not be determining an optimal pH since the buffers are at five fixed levels. However, the students will be able to assess the best pH of the options provided. pH is defined as the negative log of the hydrogen ion concentration, thus what pH really signifies is how many H⁺ ions are present around the enzymes. At concentrations either above or below the optimal pH, enzymes may change conformation which can drastically affect activity. At extremes, enzymes might actually denature.

Materials

Each laboratory group will need the following materials: Teacher’s Note: Many of the items listed below are the same as the first experiment. The only additional items are the four new buffers. If reagent tubes are re-used from the first experiment, many of the volumes will be in excess of what is listed below. Again stress to the students that overuse of reagents will lead to shortages later on.

a. 1M Acetate pH 5: 0.10 ml (about 3 drops)
b. 1M Citrate pH 6: 0.10 ml (about 3 drops)
c. 1M Phosphate pH 7: 0.10 ml (about 3 drops)
d. 1M Tris pH 8: 0.10 ml (about 3 drops)
e. 1M Tris pH 9: 0.10 ml (about 3 drops)
f. Water: 1 ml (about 25 drops)
g. PMS/INT/NAD: 0.4 ml (about 12 drops)
h. Substrate (Lactic Acid): 0.4 ml (about 12 drops)
i. Diluted Enzyme Concentrate: 0.4 ml (about 12 drops)
j. Stop Reagent: 0.25 ml (about 8 drops)
k. Reaction Tubes - 5
l. Droppers – 10 Teacher’s Note: With the exception of the enzyme pipette from the first experiment, the transfer pipettes saved from the first experiment can be used again.
m. Test Tube Rack

Method

1. You will be running enzyme reactions with five different pH buffers so you can see how the different buffers affect the reaction. Each dropper should be used with only one reagent/ingredient. Mixing up the droppers will cause contamination and cause incorrect results. **TAKE CARE SO THAT YOU DON’T CONTAMINATE THE DIFFERENT TUBES.** Label five reaction tubes #1 through #5. Teacher’s Note: This set of reactions is very similar to the first experiment, except that the variation is in pH.

2. Add 2 drops of lactic acid and 2 drops of PMS/INT/NAD to all 5 reaction tubes.

3. Add 2 drops of buffer to the tubes as follows:
   
   Tube 1 - Acetate Buffer, pH 5
   Tube 2 - Citrate Buffer, pH 6
   Tube 3 - Phosphate Buffer, pH 7
   Tube 4 - TRIS Buffer, pH 8
   Tube 5 - TRIS Buffer, pH 9

4. This is a timed reaction which is halted by the addition of Stop Reagent. Have the Stop Reagent ready and at hand so that you can efficiently add it to the reactions to stop them.

5. Add 2 drops of the enzyme, lactate dehydrogenase, to each tube (work efficiently). Shake the tubes gently to mix the reagents. Place the reaction tubes into the incubator or water bath. Start timing the reaction.

6. After five minutes, examine the tubes. If noticeable color has developed, continue to step 7, otherwise continue incubating the tubes for five additional minutes. **Teacher’s Note:** Color doesn’t have to be real dark, but rather sufficient color needs to develop in order to differentiate between the different reactions.

7. Add 1 drop of Stop Reagent into each test tube and mix to halt the reaction. Remove the vials from the incubator/water bath. **Teacher’s Note:** The Stop Reagent is a dilute solution of copper sulfate (which is also used in the inhibitor experiment). Copper ions prevent the transfer of electrons between the co-factors. Copper can also interfere with the enzyme itself.

8. Score each reaction using the color key and record it on your worksheet.
9. Complete the laboratory worksheet once the data has been collected.

Worksheet Answers:

1. **Visual Optical Density:** Record your observations in the following table.

<table>
<thead>
<tr>
<th>pH (Tube #s)</th>
<th>Color (5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 5</td>
<td>0</td>
</tr>
<tr>
<td>pH 6</td>
<td>0</td>
</tr>
<tr>
<td>pH 7</td>
<td>1</td>
</tr>
<tr>
<td>pH 8</td>
<td>4</td>
</tr>
<tr>
<td>pH 9</td>
<td>9</td>
</tr>
</tbody>
</table>

2. **Graph Results Using a Bar Graph**

![Enzyme Score vs. pH of Reaction](attachment:enzyme_score_graph.png)

3. **Questions:**
   a. Which pH level gets the strongest reaction?
   The strongest enzyme reaction appears to be at pH 9. Enzyme activity also appears at pH 8 and pH 7, but the enzymes are less effective at these lower pH levels.

   b. What effect does pH have on enzyme activity?
   The pH of the enzyme reaction has a very significant effect on the activity of the enzyme. Clearly LDH is more active as pH increases.
c. What is the importance of knowing the optimal pH for an enzyme reaction?
Enzyme reactions are used widely in clinical diagnostic testing, industrial reactions, and in biomedical research. Running enzyme reactions requires that the enzymes be tested in optimal conditions. These conditions include pH in addition to other variables like temperature.