Part A: The Effect of Temperature on Catalase Activity

You have already seen how different temperatures can affect the enzyme catalase. Recall the demonstration where three different temperatures of chicken liver – frozen, room temperature, and hot – were exposed to the substrate, H$_2$O$_2$ (hydrogen peroxide). How did each sample react?

In a similar experiment, a student measured the rate of reaction at 13 different temperatures. In this case, they had a special scientific instrument called a spectrometer, which can give them the exact rate of reaction in the unit mol/L/s. (Do not worry about the meaning of this unit at this time – the numbers are the thing that matters.)

Here is the data that they gathered:

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of Reaction (mol/L/s)</td>
<td>0</td>
<td>0.2</td>
<td>0.5</td>
<td>0.8</td>
<td>1.2</td>
<td>1.8</td>
<td>2.8</td>
<td>4.0</td>
<td>4.5</td>
<td>4.0</td>
<td>3.2</td>
<td>2.0</td>
<td>0</td>
</tr>
</tbody>
</table>

Plot the data points on the following grid and draw a smooth curve that models the data. Use a curve of best fit.

**Effect of Temperature on Enzyme Reaction**
**Analysis:**

1. Based on your graph, what was the optimum temperature for this enzyme? ____________

2. Explain why the rate of reaction *increased* before reaching optimal temperature. Include the terms: enzyme, substrate, energy, collision, active site, and orientation.

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   __________________________________________________________________________

3. Explain why the rate of reaction *decreased* after reaching optimal temperature. Include the terms enzyme, shape, active site, denature, lock, key, and substrate.

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**Part B: The Effect of pH on Catalase Activity**

**Set-up**

1. Prepare the enzyme solution/slush in either of the following ways:
   a) blend up a source of catalase – chicken or beef liver, peeled potato, etc. – with some distilled water. Use cheese cloth to filter the mixture. The filtrate (liquid) is what you will use.
   b) add some distilled water to instant yeast. Mix well.

2. Prepare a line-up of 5-14 medium-sized beakers. Add the same amount of enzyme to each. Add an equal amount of distilled water to one of the beakers. To the rest of the beakers, add an equal amount of solutions with different pHs. Eg. Vinegar, HCl, NaOH, baking soda solution, cleaning products, lemon juice, etc.

3. For each of the enzyme beakers, prepare a small beaker containing the substrate, hydrogen peroxide (H₂O₂). Make sure they all contain equal amounts of solution.

4. Prepare a pH indicator – litmus paper, red cabbage indicator strips, etc. – for each of the enzyme\substrate beaker pairs.

5. Write the numbers 0-10 on 11 sticky notes and place them in a line across a workbench. Leave about a foot between each one.

**Materials**

- Source of catalase
- Distilled water
- Cheese cloth
- Paper towel (just in case)
- 5-14 medium-sized beakers
- 5-14 small beakers
- pH indicator strips
- Tape
- Sticky notes
- Variety of solutions with different pHs.
- 3% Hydrogen Peroxide

**Safety Precautions**

- Wear gloves and safety eyewear during set-up and demonstration (students, too)
- Some of the chemicals used are corrosive. Avoid any contact with skin, eyes, or clothes. Flush spills on skin immediately with cool water.
- Do NOT mix any of the enzyme solutions together.
- Do not taste any substances in the lab.
**Enzyme Assay**

1. Dip a pH indicator into one of the enzyme beakers. Tape the indicator to the beaker. Repeat this process for the remaining catalase slushies.

2. With the students’ help, empty the contents of every substrate beaker into the corresponding enzyme beaker – *all at the same time*.

3. Very quickly but carefully, work together to line up the beakers from least reactive to most reactive. Then, rate the vigorousness of the bubbling from 0 (no reaction) to 10 (very vigorous reaction). Place the beakers in the appropriate spot on the number line. (If you rate the rate of reaction as 4.5, the beaker should be placed in between 4 and 5 on the number line).

4. Use a pH indicator colour chart to determine the pH of each of the enzyme slushies. Record the data below. You will only fill in as many columns as there are beakers.

<table>
<thead>
<tr>
<th>Beaker</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
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<td></td>
</tr>
<tr>
<td>Rate of Reaction (0-10)</td>
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</tbody>
</table>

Plot the data points on the following grid and draw a *smooth* curve that models the data. Use a curve of *best fit*.

**Effect of pH on Enzyme Reaction**

![Graph showing the effect of pH on enzyme reaction](image-url)
**Analysis:**

1. Based on your graph, what was the optimum pH for this enzyme? ______________

3. What are the bubbles you observe when a substance containing catalase is added to hydrogen peroxide? Describe in detail what is happening. Include a chemical equation.

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4. The pH scale ranges from 0-14, with a pH of 7 being neutral. A pH below 7 is acidic, and contains positively charged hydrogen ions, \( H^+ \). A pH above 7 is basic, and contains negatively charged hydroxide ions, \( OH^- \). Recall that enzymes are proteins made up of amino acids, some of which are positively charged and some of which are negatively charged. Remembering that positive and negative particles attract each other and have a tendency to bond to each other, come up with a good explanation as to why adding an acid or base to an enzyme could effect how it works. Include the words: positive, negative, bond, uncoil, denature, ions, amino acids, acid, base, inhibit, structure, function, substrate, active site.

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5. A source of error in a lab is any part of it which could cause inaccuracies in the analysis. Consider the following example. If two different people measure the length of an elastic band at different times, they may get different results because of a few factors including: the elastic may get stretched a different amount by each person, and the elastic may be at different temperatures changing its properties. Identify three possible sources of error in this enzyme lab.

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