Introduction

REVIEW ON ENZYME

Enzyme

• What is enzyme?
  – Mostly protein
  – Biological catalyst
  – Catalyst speeds up chemical reaction without being used up or altered.
  – Binds specific substrate only at an active site
Specificity of active site & substrate

Catalytic Reaction of Catalase

\[ 2H_2O_2 + \text{catalase} \rightarrow 2H_2O + O_2 + \text{catalase} \]

Watch at home

• Animations of Enzyme Action:
  
  http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

  http://www.youtube.com/watch?v=V4OPO6JQ

Laboratory Objectives

• Investigate Catalase Activity Based on Scientific Method:
  
  1. Observation (We know that):
     • Catalase catalyzes (speeds up) the biochemical reaction to break down hydrogen peroxide into oxygen and water.
     • Catalase works best at an optimum temperature and pH.
  2. Question:
     • How do the following factors affect the rate of catalase activity (i.e., the speed of reaction)?
       » Enzyme conc.
       » Substrate conc.
       » pH
  3. Hypotheses:
     • The higher conc. of enzyme or substrate increases the rate of catalase activity.
     • At an optimum pH, the rate of catalase activity is the highest.
4. Predictions:
• If the higher conc. of enzyme or substrate increases the rate of catalase activity, the product \(O_2\) will be formed faster, and thus the filter disc will float faster.
• At an optimum pH, the rate of catalase activity is the highest.
• If the rate of catalase activity is the highest at an optimum pH, the filter disc will float the fastest at the optimum pH.

5. Conducting Controlled Experiment:
• We will test the hypotheses by measuring time for a filter paper disc to float at different concentrations of catalase and \(H_2O_2\), and different pH’s.

Organize & Check Items
- Water for enzyme dilution
- Substrate dilution buffer
- Vial of catalase
- Marker & Forceps
- Hole puncher
- Substrate \((H_2O_2)\)
- Liquid Waste Container
- Kimwipes

- Catalase and \(H_2O_2\) to be provided at the beginning of the lab to equilibrate to room temperature.
- Avoid cross-contamination.
- Keep the marker tip-side down.
- Empty and return the following bottles to lab instructor:
  - substrate bottle, dilution buffer bottle, enzyme vial.
- Organize remaining items as shown after exercise.

Front Supply Table
- Left
  - pH 3
  - pH 5
  - pH 7
- Right
  - Fresh solution distribution for instructor
  - Filter Paper for disc preparation
  - Disposable transfer pipettes
  - Disposable Plastic cups

- Buffered \(H_2O_2\) substrates are ready for reaction.
  - (Contains both \(H_2O_2\) & pH buffer.)
- Use 10 ml of each pH

- Ask lab instructor for refill of dilution buffer, substrate or enzyme.
- Use fresh cups for each experiment.
Activity A: Enzyme Concentration

<table>
<thead>
<tr>
<th>Enzyme Conc. (Units/ml)</th>
<th>Dil. Buffer to add (ml)</th>
<th>Catalase to add (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>75</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Prepare 5 cups of catalase at different conc.
Prepare 1 cup of Substrate (H₂O₂)

Pour ~20 ml of H₂O₂ Out of brown bottle without dilution

Measuring Reaction Time

- Use forceps to grab a disc at an edge. DO NOT hold with fingers. DO NOT deform the disc.
- Dip one filter disc at a time
- Blot excess enzyme solution on a piece of Kimwipes. Be consistent so that each disc contains similar amt. of enzyme
- Measure time at one catalase conc. at a time
- Calculate the rate of reaction using the following formula:

\[
\frac{1}{\text{Time required to float}}
\]
Activity B: Substrate Concentration

Prepare 4 cups of Substrate (H₂O₂) at different conc.

<table>
<thead>
<tr>
<th>H₂O₂ Conc. (%)</th>
<th>4% H₂O₂ to add (ml)</th>
<th>Dilution Buffer to add (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>1.0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2.0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>4.0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Activity C: Effect of pH

Obtain 4 cups of buffered Substrate (H₂O₂) of different pH

Measuring Reaction Time

1. Use forceps to grab a disc at an edge. DO NOT hold with fingers. DO NOT deform the disc.
2. Dip one filter disc at a time
3. Blot excess enzyme solution on a piece of Kimwipes. Be consistent so that each disc contains similar amt. of enzyme.
4. Measure time at one catalase conc. at a time
5. Calculate the rate of reaction using the following formula:

\[
\frac{1}{\text{Time required to float}}
\]
Completion of the lab

- Empty & dispose of used plastic cups in the trash can.
- Empty & return the catalase tube, bottles for 4% H₂O₂ and dilution buffer so that your lab instructor can refill them for the next lab session. Not required to wash unless they are contaminated with enzyme.
- Empty, rinse & return the Liquid Waste Container on your lab table.

Organize & Check Items

- Catalase and H₂O₂ to be provided at the beginning of the lab to equilibrate to room temperature.
- Avoid cross-contamination.
- Keep the marker tip-side down.
- Empty and return the following bottles to lab instructor:
  - substrate bottle, dilution buffer bottle, enzyme vial.
- Organize remaining items as shown after exercise.