BIOL 1011

Unit 04:
Enzyme-Action of Catalase

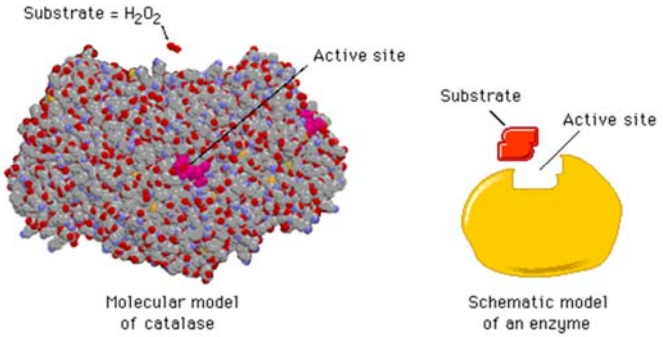
Introduction

REVIEW ON ENZYME

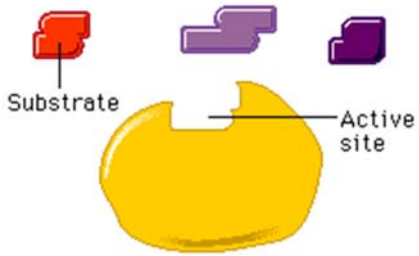
Enzyme

- 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**

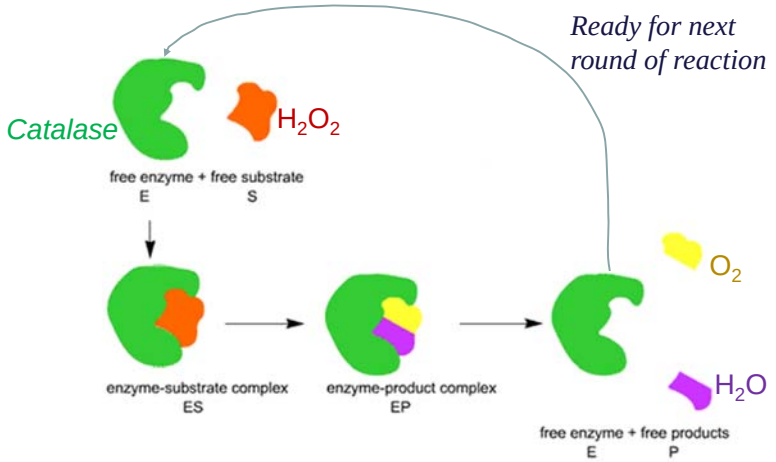
Enzyme: Active site & Substrate



Specificity of active site & substrate



Catalytic Reaction of Catalase



$2H_2O_2$ + catalase → $2H_2O + O_2$ + catalase

Watch at home

- Animations of Enzyme Action:

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

How Enzymes Work

http://www.youtube.com/watch?v=V4OPO6JQLOE

YouTube

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.**

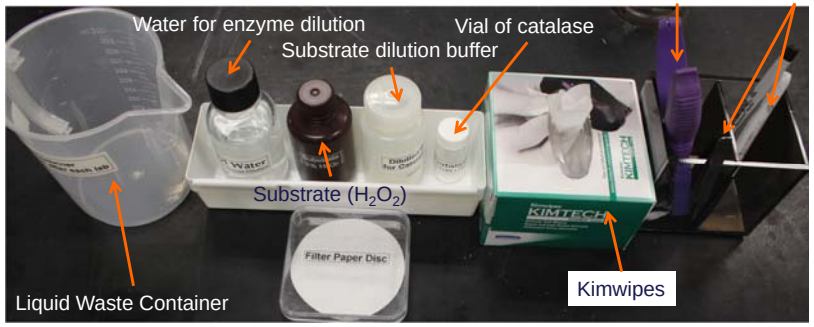
Laboratory Objectives- cont'd

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.**

Controlled Experiments

MEASURING THE RATES OF ENZYME REACTIONS

Organize & Check Items

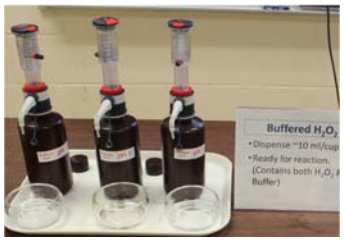


- *Catalase and H_2O_2 to be provided at the beginning of the lab to equilibrate to room temperature.*
- Avoid cross-contamination.
- Keep the marker tip-side down.
- Empty and return the following bottles to lab instructor:
  - ✓ substrate bottle, dilution buffer bottle, enzyme vial.
- Organize remaining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

Front Supply Table

- Left

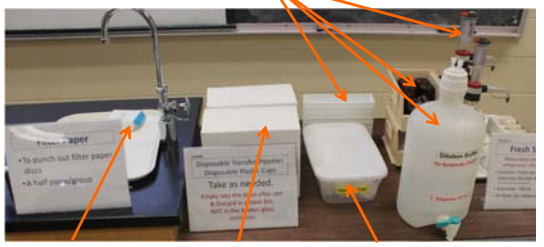
pH 3 pH 5 pH 7



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

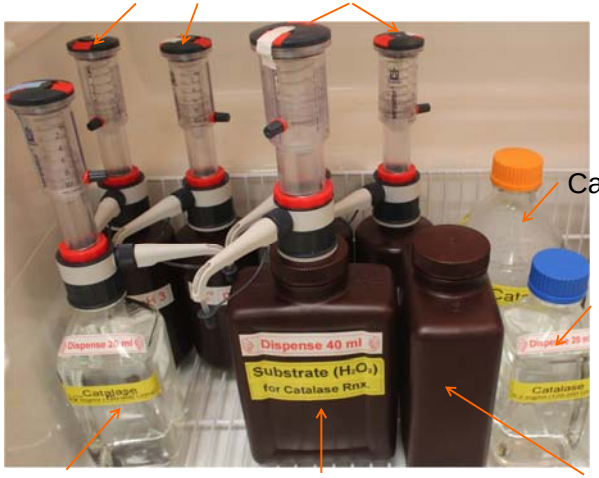
- Right

Fresh solution distribution for instructor



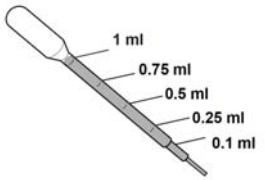
- ✓ Ask lab instructor for refill of dilution buffer, substrate or enzyme.
- ✓ Use fresh cups for each experiment.

In Refrigerator (for Instructor only)

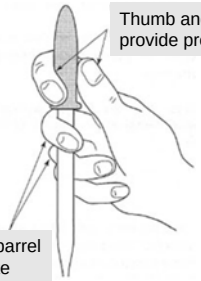


Using a transfer pipette (Pasteur pipet)

Volume marks on a transfer pipette (Pasteur pipet)



Holding a transfer pipet



Activity A:

Enzyme Concentration

Enzyme Conc. (Units/ml)	Dil. Buffer to add (ml)	Catalase to add (ml)
0	4	0
25	3	1
50	2	2
75	1	3
100	0	4

Prepare 5 cups of catalase at different conc.

Prepare 1 cup of Substrate (H_2O_2)

Pour ~20 ml of H_2O_2 Out of brown bottle without dilution

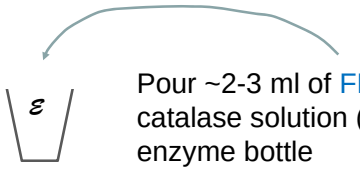
Measuring Reaction Time

- 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 1 cup of Enzyme



Pour ~2-3 ml of FRESH undiluted catalase solution (100 U/ml) out of enzyme bottle

H_2O_2 Conc. (%)	4% H_2O_2 to add (ml)	Dilution Buffer to add (ml)
0	0	8
1.0	2	6
2.0	4	4
4.0	8	0

Prepare 4 cups of Substrate (H_2O_2) at different conc.

Measuring Reaction Time

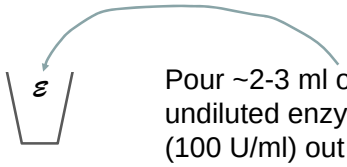
(same as in Activity A)

- 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 C: Effect of pH

1 cup of Enzyme



Pour ~2-3 ml of FRESH undiluted enzyme solution (100 U/ml) out of blue capped tube

- Obtain ~10 ml of each buffered substrate in separate cups from brown square bottles on the front supply table.
- The buffered H_2O_2 solutions are ready for reaction. DO NOT add any solution; just place catalase-soaked filter paper discs.

Obtain 4 cups of buffered Substrate (H_2O_2) of different pH

Measuring Reaction Time

(same as in Activity A & B)

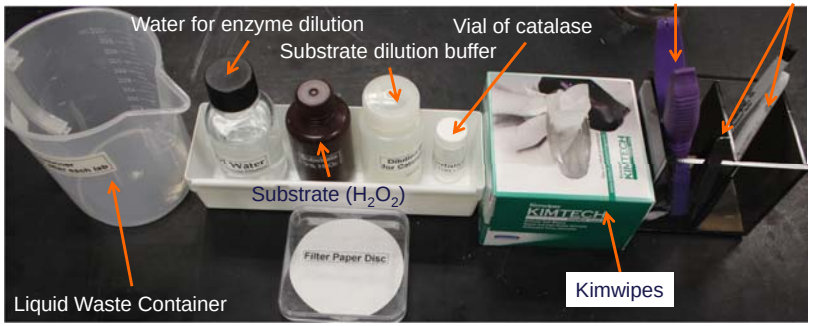
- Use forceps to grab a disc at an edge.
  DO NOT hold with fingers.
  DO NOT deform the disc.
- Dip one filter disc at a time
- Blot excess enzyme solution on a piece of Kimwipes. Be consistent so that each disc contains similar amt. of enzyme
- Measure time at one catalase conc. at a time
- Calculate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

$$\frac{1}{\text{Time required to float}}$$

Completion of the lab

- Empty & dispose of used plastic cups in the trash can.
- Empty & return the catalase tube, bottles for 4% H_2O_2 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



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