Lab 2. Enzyme Action—Effect of Enzyme Concentration, Temperature and pH on Catalase Activity

Prelab Assignment

Before coming to lab, read carefully the introduction and the procedures for parts I-III, and then answer the prelab questions at the end of this lab handout. Hand in the prelab assignment just *before* the start of your scheduled lab period.

Goals of this Lab

After completing this lab exercise you should be able to...

- Use a computer and pressure sensor to measure the initial rate of decomposition of hydrogen peroxide by the enzyme catalase at various enzyme concentrations, pH, and temperature values.
- Define enzyme, catalyst, active site, substrate, activation energy, product, denaturation and explain how enzymes function.
- Predict the effects of varying environmental conditions such as pH and temperature on enzyme structure and activity.
- Predict the effects of varying enzyme and substrate concentrations on enzyme activity.
- Propose hypotheses, make predictions based on hypotheses, test hypotheses, and evaluate experimental data to see if it supports or falsifies the hypotheses being tested.
- Practice scientific persuasion and communication by constructing and interpreting graphs of enzyme activity.

Introduction and Background Information

Life without enzymes is unimaginable. In the absence of enzymes, the energy required by your muscles simply to open your lab manual would take years to accumulate. Due to the action of enzymes, the thousands of chemical reactions occurring in your cells at this very moment are being completed in a fraction of a second rather than the years or decades that would otherwise be required. Without enzymes, for example, the energy required for you to simply blink your eye would take years to be produced.

Enzymes are biological catalysts. A catalyst speeds up a chemical reaction by lowering the ***activation energy*** of a reaction, the energy needed to begin a reaction. Enzymes are organic catalysts made in all or in part of protein. Like all catalysts, enzymes are not destroyed nor altered by the reaction. Enzymes are extremely efficient as a single enzyme molecule may be used over and over again. One enzyme molecule may catalyze a specific chemical reaction thousands of times every second. Because of this high rate of activity, only a very small amount of enzyme is needed to act on a relatively large amount of ***substrate***, the substance the enzyme acts upon.

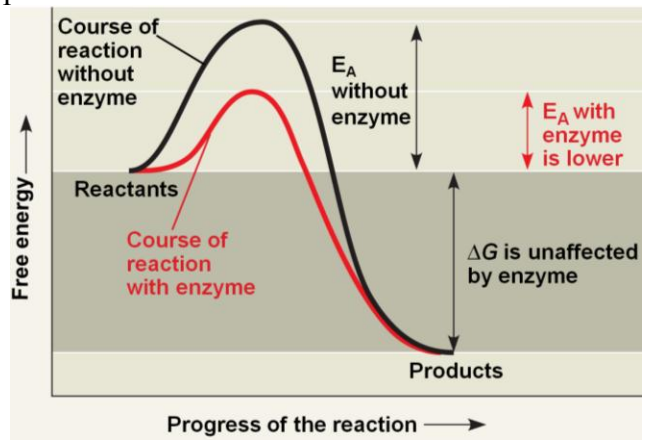
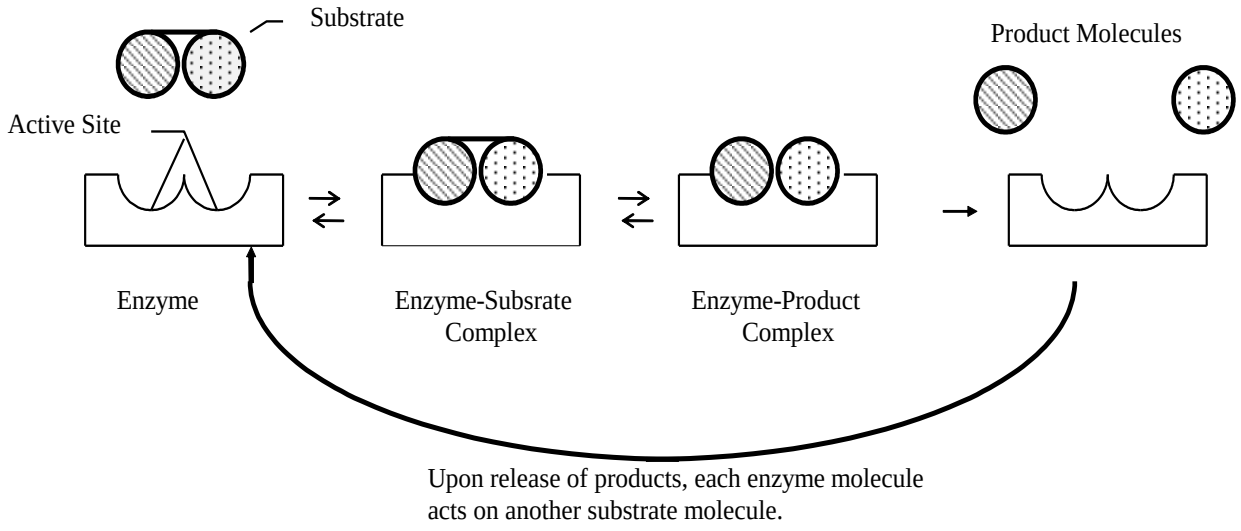


Figure 1. Enzymes the rate of a chemical reaction by decreasing the activation energy, the energy required to initiate a reaction.

In an enzyme catalyzed reaction, a ***substrate*** molecule first interacts with the ***active site*** of the enzyme, forming an ***enzyme-substrate complex*** (ES). The substrate is then converted into one or more products and then released from the enzyme. The interaction between the substrate and the active site reduces the ***activation energy*** (the minimum kinetic energy required by the reactant(s) for a reaction to occur) of the reaction, thereby increasing the fraction of molecules with sufficient kinetic energy to react. As a consequence, the rate of reaction increases dramatically. Enzyme catalyzed reactions may proceed from a hundred thousand to 10 million times faster than they would without the enzyme present!

Enzyme + Substrate ⇌ E-S Complex ⇌ E-P Complex → Enzyme + Product

A. Catabolic Reaction. Enzymes can facilitate the breakdown of larger molecules into simpler substances.



B. Anabolic Reaction. Enzymes can facilitate the synthesis of larger molecules from simpler substances.

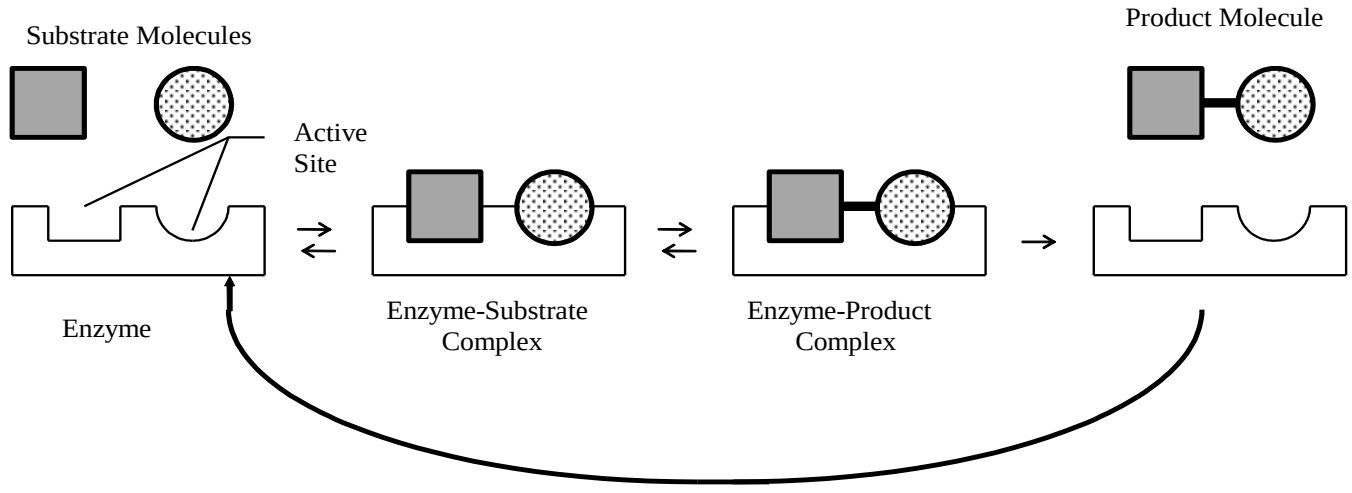


Figure 2. An enzyme can only act on substrate molecules that can bind to its active site. Very slight changes in the structure of the active site will alter the catalytic functioning of an enzyme.

Common ways enzymes lower the activation energy to accelerate the Rate of Reaction:

1. Binding of the substrate to the enzyme's active site may result in the straining of one or more chemical bonds in the substrate, thus increasing the probability of bond(s) breaking. (Catalase and peroxidase destabilize the oxygen-oxygen and oxygen-hydrogen bonds in H_2O_2, thus facilitating its decomposition.)
2. An enzyme may hold the substrate molecule(s) in such a way to bring reacting parts of the molecule(s) close to one another to increase the probability of a reaction. (E.g. The synthesis of the disaccharide sucrose (table sugar), by combining the monosaccharides glucose and fructose.)
3. Side groups of the amino acids within the active site of the enzyme may act as proton donors or acceptors to help promote acid-base reactions involving the substrate.
4. An enzyme may temporarily react with the substrate molecule to form an unstable intermediate that the readily undergoes a second reaction that generates the products along with an unchanged enzyme ready to undergo further catalysis reactions

Factors that Influence Enzyme Activity

Enzyme activity is influenced by many factors. Both the temperature and the pH at which enzymes function are extremely important. Most organisms have a preferred temperature and pH range in which they survive, and their enzymes usually function best within very narrow temperature and pH ranges. If the environment of the enzyme is too acidic, basic, or hot, the activity of the enzyme may be altered due to a change in the three-dimensional shape of the enzyme. ***Denaturation***, the unraveling or structural changes of an enzyme, may be temporary or permanent depending on the degree of the environmental change. In either case, a denatured enzyme no longer has the shape necessary to interact with the substrate effectively to lower the activation energy.

In this experiment you will investigate enzyme action on ***hydrogen peroxide***, H_2O_2. H_2O_2 is a natural byproduct of aerobic metabolism. H_2O_2 quite toxic to most organisms since its production leads to the formation of ***hydroxyl free radicals***, the most damaging of all free radicals since they react with and damage all biological molecules they collide with, including DNA. Fortunately, most of the H_2O_2 produced is detoxified by converting it to harmless oxygen gas and water, as follows:

$$\mathbf{2\ H_2O_2}\ \textit{(aq)} \rightarrow \mathbf{2\ H_2O}\ \textit{(l)} + \mathbf{O_2}\ \textit{(g)}$$

Although this reaction occurs spontaneously, enzymes increase the rate considerably. At least two different enzymes are known to catalyze this reaction: ***catalase,*** found in all animals (including most tissues in the human body) and protists, and ***peroxidase***, found in plants. A great deal can be learned about enzymes by studying the rates of enzyme-catalyzed reactions.

In this experiment, you will measure the rate of catalase activity under various conditions, such as different concentrations of enzyme, pH values, and temperatures. The rate of a chemical reaction may be determined by either measuring the rate at which the substrate disappears, or by determining the rate of appearance of the product. In this experiment the rate of decomposition of H_2O_2 will be determined by measuring the appearance of oxygen by monitoring pressure of oxygen gas formed as H_2O_2 is destroyed. If a plot of pressure vs. time is made, it should appear similar to that of Figure 3.

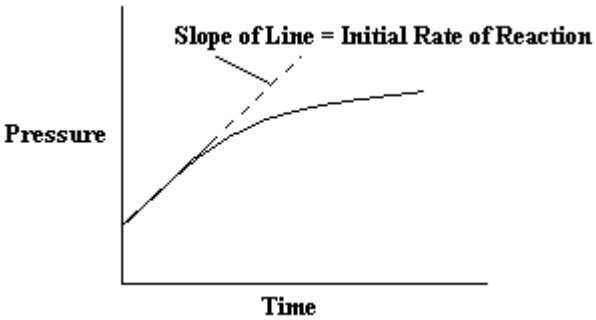


Figure 3. Pressure as a function of time during the decomposition of H_2O_2. Note that at the start of the reaction (i.e. t = 0) there is no product formation, and the pressure is the same as the atmospheric pressure. After a short time, oxygen accumulates at a rather constant rate. The slope of the curve at this initial time is constant and is called the ***initial rate***. As the substrate, hydrogen peroxide, is decomposed, its concentration decreases which results in fewer enzyme-substrate collisions; hence ***the rate of O_2 production decreases over time***.

Materials

Per Group of 2 Students:
- Computer
- Serial Box Interface
- Vernier Biology Gas Pressure Sensor with rubber stopper assembly
- Two - 10 mL graduated cylinders
- 250-mL beaker of tap water at room temp.
- 600 or 1000 mL beaker
- Dropper pipettes
- Test tube rack
- Five - 18 x 150 mm test tubes

For Groups Doing Part II
- Thermometer
- Turkey baster
- Thermostatically controlled water bath set at 75 ^{o}C
- Ice

For Groups Doing Part III
- Buffer solutions of pH 3, 5, 7, 9, and 11

Per Class:
- 3.0 % H_2O_2
- Catalase enzyme suspension
- Carboy containing DI water at room temperature

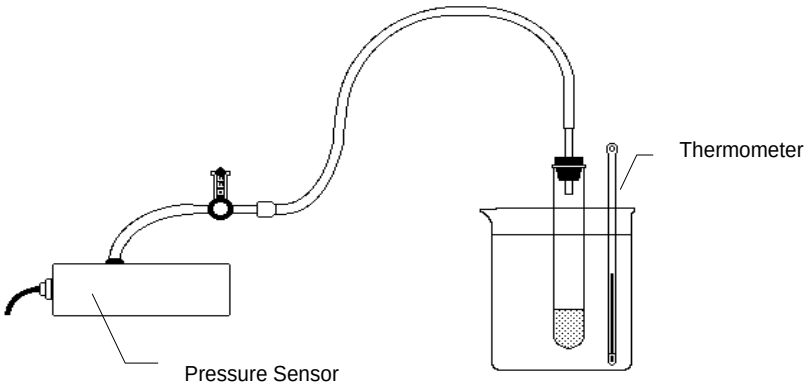


Figure 4. Experimental Set-up with reaction vessel in a water bath maintained at a constant temperature.

Important considerations:

i.) To ensure an accurate measurement of pressure while the H_2O_2 is decomposed by catalase, the test tube must be *swirled at a steady rate* to facilitate the liberation of O_2 gas from the solution.

ii.) The test tube should be submerged in the water bath as far as possible to keep the entire contents at the temperature of the water bath.

iii.) There is a valve attached to the rubber stopper (not shown above) that should be open while attaching the assembly to the test tube, and then closed just before the start of data collection—see figures 6A and 6B for details.

Influence of Concentration, pH, and Temperature on the Activity of Catalase

Procedures (Perform in teams of 2)

Important Note!! To get an overview of this laboratory activity and to use your lab time efficiently read the procedures for all parts *before* attending lab. If you and your group members are not familiar with these procedures before coming to lab you will have great difficulty completing this exercise during the lab period.

Preparation of the computer for Data Collection

1. Prepare the computer for data collection as follows:
   a. Connect the Gas Pressure Sensor to the Go-Link.
   b. Open *Logger Pro* within the *Biology with Vernier* folder. Now open EXP 6B Enzyme (Pressure)
   c. Verify that the vertical axis has pressure scaled from ~90 to ~130 kPa and the horizontal axis has time scaled from 0 to 3 *minutes* (not seconds!!)
   d. Check the accuracy of the pressure sensor: A pressure reading of ~ 99 to ~103 kPa should appear when the sensor is open to the atmosphere.
2. Connect the stopper assembly to the pressure sensor, making sure all connections are "finger tight" and do not leak!
3. **Adjusting the Valve to the Pressure Sensor.**
   Open the valve on the rubber stopper assembly so that it is *open to the atmosphere*. Do this by turning the handle of valve on the rubber stopper so that it is in a vertical position as in figure 6, below.

Figure 5. Open to the atmosphere. Any gas generated in the tube will be vented to the air

Figure 6. Closed to the atmosphere. Any gas generated in the tube will be contained and monitored by the pressure sensor.

Part I. Testing the Effect of Enzyme Concentration on the Rate of Reaction

Introduction

In this part of the experiment you will vary the concentration of the enzyme catalase to determine what effect the Catalase concentration has on the rate of the reaction. All trials will be performed in a water bath at room temperature.

Procedure (Perform in teams of two)

4. a. Perform steps 1-3 (Preparation of the computer for Data Collection), above.

   b. All trials will be performed in a water bath at room temperature: Prepare a water bath at room temperature by placing water from the water carboy in a 600-mL beaker. Hold the test tube as *fully submerged* in the water bath as possible as in Figure 4.

5. Place five test tubes in a rack and label them 1, 2, 3, 4, and 5. Use 10 mL graduated cylinders to add 3.0 mL of water and 3.0 mL of 3.0 % H_2O_2 to each test tube as indicated in table 1.

Important!!! Be ready to proceed immediately to steps 7 - 12 before adding the enzyme suspension to the substrate, H_2O_2, in step 6!

Table 1. Contents of test tubes 1-5

Test tube No.	Volume of 3.0 % H_2O_2 (mL)	Volume of D.I. water (mL)	Drops of Enzyme Suspension
1	3.0	3.0	2
2	3.0	3.0	5
3	3.0	3.0	10
4	3.0	3.0	15
5	3.0	3.0	0

6. Use a clean dropper pipette to add 2 drops of enzyme suspension to Test Tube 1. Be sure the enzyme does *not* fall against the sides of the test tube. ***Steps 7-10 should be completed as rapidly as possible!***

7. With the valve of the stopper assembly open (in the vertical position as in figure 5), quickly insert the stopper assembly firmly into the test tube. *Firmly* twist the stopper for an *airtight* fit.

8. Swirl the tube to thoroughly mix its contents. The reaction should begin.

Collection of Data

9. **Close the system to the atmosphere**:
   a. Close the valve on the rubber stopper assembly so that the O_2 gas produced by the reaction cannot escape. Do this by turning the handle of the valve on the rubber stopper assembly to a horizontal position as in figure 6, above.

   b. The pressure sensor will now be measuring the pressure within the test tube. **After data collection has started do not tamper with the fittings, as this will alter the pressure of the system**.

10. Begin collecting data by clicking the Start button. Gently ***swirl*** the test tube continuously while collecting data (this helps to liberate the oxygen gas from the solution and helps to keep the contents mixed well). **Monitor the temperature of the water bath. Be sure that it does not change by more than one degree.**
11. Collect data until you are certain that there is a linear relationship between the pressure and time—this usually takes less than a minute. If the pressure exceeds 130 kPa, stop the computer by clicking the Stop button. Open the air valve on the stopper assembly to avoid it popping off!
12. **Determination of the rate of Reaction.** The slope of the line on the monitor is equal to the rate of decomposition of H_2O_2 by catalase. Use the computer to calculate the slope as follows:
    a. Move the cursor to the point where the pressure values *begin* to have a linear relationship. Hold down the mouse button. Drag the cursor to the *end* of the linear section of the curve (as in fig. 7, below) and release the mouse button.
    b. Click the Linear Fit button, , to perform a linear regression. A floating box will appear with the formula for a best fit line. Click on the box and set the number of decimal places to give the slope 3 significant figures.

       Note: The equation for the line displayed on the monitor is **y = mx + b,** where…
       - **y** is the variable on the y-axis, pressure (in kPa)
       - **x** is the variable on the x-axis, time (in minutes)
       - **m** is the slope of the line = rate of reaction (in kPa/min)
       - **b** is the y-intercept (the pressure at t = 0; i.e. atmospheric pressure in kPa)

    c. Record in Table 4 of the report sheet the *slope of the line to 3 significant figures*, *m*, as the rate of H_2O_2 decomposition by catalase.
    d. Close the linear regression floating box.
    e. Share your group's data with the class by recording it on the class data sheet provided by the instructor.

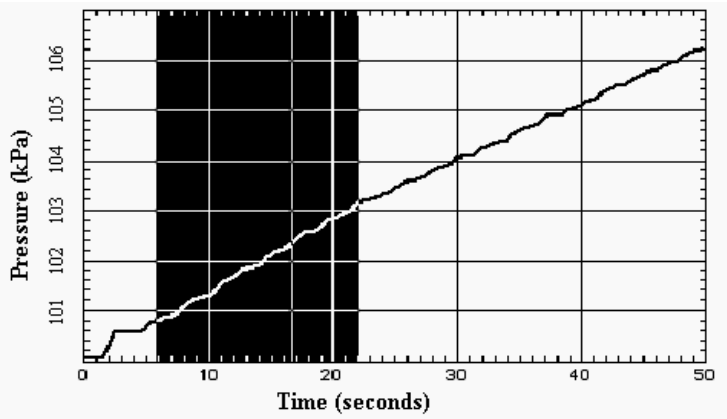


Figure 7. Determination of the rate of decomposition of H_2O_2 by catalase (i.e. the slope) from a plot of Pressure vs. Time. The data in the graph is fictitious and *not* intended to represent data obtained in this experiment!!

Important!!

Depending on the rate of the reaction with two drops of enzyme suspension, we may have to change the number of drops of enzyme suspension needed tubes 2-4!!

13. **Find the rate of enzyme activity for Test Tubes 2 – 5**:

    Be sure *not* to let the drops of enzyme suspension fall against the side of the test tube as the enzyme must be in the solution to catalyse the reaction!

    a. Add 5 drops of the enzyme solution to Test Tube 2. Repeat Steps 7 – 12.
    b. Add 10 drops of the enzyme solution to Test Tube 3. Repeat Steps 7 – 12.
    c. Add 15 drops of the enzyme solution to Test Tube 4. Repeat Steps 7 – 12.
    d. Do not add any enzyme to the solution in Test Tube 5. Repeat Steps 7 - 12.

Part II. Testing the Effect of Temperature on the Rate of an Enzyme Catalyzed Reaction

Introduction

The rate of a chemical reaction increases as temperature rises, in part because molecular velocity is increased. This means that substrate molecules collide more frequently with the active site of enzyme molecules. Generally, according to the ***Q_{10} rule***, a 10 ^{o}C rise in temperature results in a two-fold increase in the rate of a chemical reaction. However, the activity of an enzyme is dependent upon the proper three-dimensional structure, and high temperatures may irreversibly denature (alter the shape of) an enzyme. ***The optimum temperature for enzyme activity, therefore, may vary depending on the structure of the enzyme, its source, and the nature of the substrate***.

In this part of the experiment you will vary the temperature of the substrate and enzyme solutions to determine both the optimum temperature for catalase activity and if catalase follows the Q_{10} rule. As before, the decomposition of H_2O_2 by catalase will be determined by measuring the pressure of oxygen gas formed as H_2O_2 is decomposed.

Procedure (Perform in groups of two)

14. Perform steps 1-3 (Preparation of the computer for Data Collection), above, and then prepare the four test tubes as directed in step 15, below.

15 Obtain 4 test tubes and use 10 mL graduated cylinders to add 3.0 mL of 3.0 % H_2O_2 and 3.0 mL of D.I. water to each tube, as shown in Table 2. These tubes will be incubated in water baths at 10.0 ^{o}C (tube 1), 20.0 ^{o}C (tube 2), 37.0 ^{o}C (tube 3), and 75.0 ^{o}C (tube 4). Label each tube with its tube number and the temperature of the water bath that it will be placed in. Record the *actual* temperature of each water bath in table 2, below, and table 4 of the report sheet.

Table 2. Contents and temperatures for test tubes 1-4			
Test tube No.	**Temperature**	**Volume of 3.0 % H_2O_2 (mL)**	**Volume of D.I. water (mL)**
1	10.0 °C	3.0	3.0
2	20.0 °C	3.0	3.0
3	37.0 °C	3.0	3.0
4	75.0 °C	3.0	3.0

16. **Measurement of the rate of reaction at 10.0 °C**
    a. Prepare a 10.0 °C water bath by placing ice and water in a 600-mL beaker. See Figure 4. Record the *actual* temperature of the water bath in Table 4 of the report sheet and take measures to keep the temperature constant throughout the test.
    b. Place Test Tube 1 in the cold water bath until the temperature of its contents reaches 10.0 °C (about 5-10 minutes).
    c. Obtain a clean test tube and label it Tube 1E ("E" stands for enzyme). Add about 1 to 2 ml of enzyme solution to tube 1E and then place it in the 10.0 °C water bath until the enzyme solution reaches 10.0 °C (about 5-10 minutes).

Important!!

- Depending on the rate of the reaction in part 1, we may have to change the number of drops of enzyme suspension needed for the following steps—every group in the class will use the same number of drops of enzyme suspension for all trials at all temperatures!
- Be ready to proceed immediately to steps 7 - 12 before adding the enzyme solution to the substrate, H_2O_2, in the next step! Be sure *not* to let the enzyme fall against the side of the test tube.

    d. When ready to proceed *immediately* to steps 7 - 12, add *5 drops* of the enzyme (see important note, above!!) solution from Tube 1E to Test Tube 1 and Repeat Steps 7 – 12.

17. **Measurement of the rate of reaction at 20.0 °C**
    a. Prepare a 20.0 °C water bath by placing water from the water carboy in a 600-mL beaker. See Figure 4. Record the actual temperature of the water bath in Table 4 of the report sheet. Check that the water bath temperature remains constant throughout the test.
    b. Place Test Tube 2 in the water bath until the temperature of its contents reaches 20.0 °C (about 5-10 min.).
    c. Obtain a clean test tube and label it Tube 2E. Add about 1 to 2 ml of enzyme solution to tube 2E and then place it in the 20.0 °C water bath until the enzyme solution reaches 20.0 °C (about 5-10 minutes).
    d. When ready to proceed *immediately* to steps 7 - 12, add *5 drops* of the enzyme solution from Tube 2E to Test Tube 2 and Repeat Steps 7 – 12.

18. **Measurement of the rate of reaction at 37.0 °C**

    a. Prepare a 37.0 °C water bath by placing hot tap water in a 600-mL beaker. Record the actual temperature of the water bath in Table 4 of the report sheet and take measures to keep the temperature constant throughout the test. See Figure 4.
    b. Place Test Tube 3 in the 37.0 °C water bath until the temperature of its contents reaches 37.0 °C. (about 5-10 minutes).
    c. Obtain a clean test tube and label it Tube 3E. Add about 1 to 2 ml of enzyme solution to tube 3E and then place it in the 37.0 °C bath until the enzyme solution reaches 37.0 °C (about 5-10 minutes).
    d. When ready to proceed *immediately* to steps 7 - 12, add *5 drops* of the enzyme solution from Tube 3E to Test Tube 3 and repeat Steps 7 – 12.

19. **Measurement of the rate of reaction at 75.0 °C**

    a. Place Test Tube 4 in the thermostatically controlled 75.0 °C water bath in the back of the room until the temperature of its contents reaches 75.0 °C (about 5-10 minutes).
    b. Obtain a clean test tube and label it Tube 4E. Add about 1 to 2 ml of enzyme solution to tube 4E and then place it in the thermostatically controlled 75.0 °C water bath until the enzyme solution reaches 75.0 °C (about 5-10 minutes).
    c. Prepare a 75.0 °C water bath by placing water from the thermostatically controlled 75.0 °C water into a 600-mL beaker. Record the actual temperature of the water bath in Table 4 of the report sheet and take measures to keep the temperature constant throughout the test.
    d. When ready to immediately proceed to steps 7 - 12, add *5 drops* of the enzyme solution from Tube 4E to Test Tube 4 and Repeat Steps 7 – 12.

Part III. Testing the Effect of pH on the Rate of an Enzyme Catalyzed Reaction

Introduction

The pH of the solution an enzyme is in can influence the three-dimensional shape of the enzyme. Every enzyme has an optimum pH at which it is most active. Most enzymes function best in solutions that are near neutral (pH 6-8), but several work in the more basic (pH 8-12) or the more acidic range (pH 2-6). In this experiment you will determine the optimum pH for the activity of catalase.

Procedure (Perform in groups of two)

20. a. Perform steps 1-3 (Preparation of the computer for Data Collection), above.

    b. All trials will be performed in a water bath at room temperature: Prepare a water bath at room temperature by placing water from the water carboy in a 600-mL beaker. See Figure 4.

21. Place five clean test tubes in a rack and label them pH 3, pH 5, pH 7, pH 9, and pH 11. Use 10 mL graduated cylinders to add 3.0 mL of each pH buffer to each test tube, as in Table 3.

Table 3. Contents and pH values for test tubes 1-5			
Test Tube	**pH of buffer**	**Volume of buffer (mL)**	**Volume of 3.0 % H_2O_2 (mL)**
1	pH 3	3.0	3.0
2	pH 5	3.0	3.0
3	pH 7	3.0	3.0
4	pH 9	3.0	3.0
5	pH 11	3.0	3.0

Important Notes!!

- Depending on the rate of the reaction in part 1, we may have to change the number of drops of enzyme suspension needed for the following steps—every group in the class will use the same number of drops of enzyme suspension for all trials at all pH values!
- Be ready to proceed immediately to steps 7 - 12 before adding the enzyme solution to the substrate, H_2O_2, in the next step! Be sure *not* to let the enzyme fall against the side of the test tube.

22. Add 5 drops of enzyme suspension to 3.0 mL pH 3 buffer and wait a minimum of 5 minutes for the enzyme to be affected by the buffer, then add 3.0 mL 3.0% H_2O_2 and immediately do steps 7 -12.
23. In the tube labelled pH 5, add 5 drops of the enzyme solution and wait at least 5 minutes to allow the enzyme to be affected by the buffer. Now add 3.0 mL 3.0% H_2O_2 and immediately do steps 7 -12
24. In the tube labelled pH 7, add 5 drops of the enzyme solution and wait at least 5 minutes. Now add 3.0 mL 3.0% H_2O_2 and immediately do steps 7 -12
25. In the tube labelled pH 9, add 5 drops of the enzyme solution and wait at least 5 minutes. Now add 3.0 mL 3.0% H_2O_2 and immediately do steps 7 -12
26. In the tube labelled pH 11, add 5 drops of the enzyme solution and wait at least 5 minutes. Now add 3.0 mL 3.0% H_2O_2 and immediately do steps 7 -12

After completing Today's Lab Activity...

- Clean your glassware and lab table.
- Return your cleaned equipment to the lab cart.
- Ensure that the lab cart and work areas are neat and clean.
- Record your group's results on the class data sheet at the front of the room.
- Obtain from the class data sheet the data for all parts of today's lab.

When I die, I want to go peacefully like my Grandfather did, in his sleep— not screaming, like the passengers in his car.

Lab 2 Report Sheet - Biol 211 **Name** _____
Enzyme Action: **Group Number** _____ **Date** _____
Testing Catalase Activity

Results

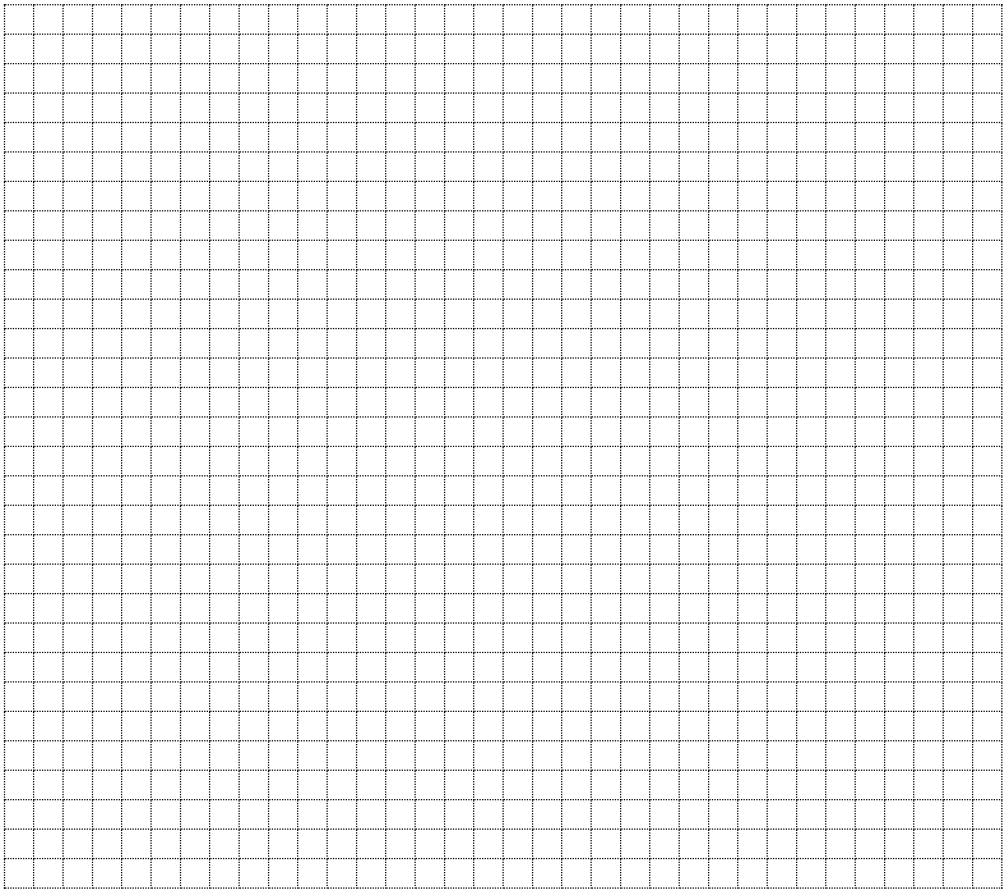
Table 4. Data for Parts I - III		
Part I. Effect of enzyme concentration on the rate of H_2O_2 decomposition		
Test tube label	**Rate (kPa/min)**	**Misc. Notes and Comments**
Tube 1: 2 Drops Enzyme		
Tube 2: 5 Drops Enzyme		
Tube 3: 10 Drops Enzyme		
Tube 4: 15 Drops Enzyme		
Tube 5: No Enzyme		
Part II. Effect of temperature on the rate of H_2O_2 decomposition		
Test tube label	**Rate (kPa/min)**	**Misc. Notes and Comments**
Tube 1: 10.0°C		
Tube 2: 20.0°C		
Tube 3: 37.0°C		
Tube 4: 75.0°C		
Part III. Effect of pH on the rate of H_2O_2 decomposition		
Test tube label	**Rate (kPa/min)**	**Misc. Notes and Comments**
Tube 1: pH 3		
Tube 2: pH 5		
Tube 3: pH 7		
Tube 4: pH 9		
Tube 5: pH 11		

Analysis of the Results

Part I. Testing the Effect of Enzyme Concentration on Enzyme Activity

Plot of Rate of Reaction vs. Enzyme Concentration

1. Using *Excel* or by hand, make a graph of the rate of enzyme activity vs. enzyme concentration. Recall that the dependent variable is plotted on the y-axis, the independent variable on the x-axis. Label the graph fully and give it a proper title.



2. Restate the hypothesis you made for this part of the experiment. Does the data support or refute your hypothesis? Summarize what can be concluded from the data. *Quote specific data* to support the conclusions that you make. Your summary should include a discussion on *how* changing the catalase concentration affects the rate of the decomposition of hydrogen peroxide and *why* changing the catalase concentration affects the rate of reaction.

3. Use the experimental results to predict the effect on the rate of reaction if one increases the amount of enzyme to 20 drops. How about 100 drops? Explain your reasoning and quantify your response.

4. No enzyme was added to tube 5. What was the purpose of conducting the experiment using tube 5? Explain.

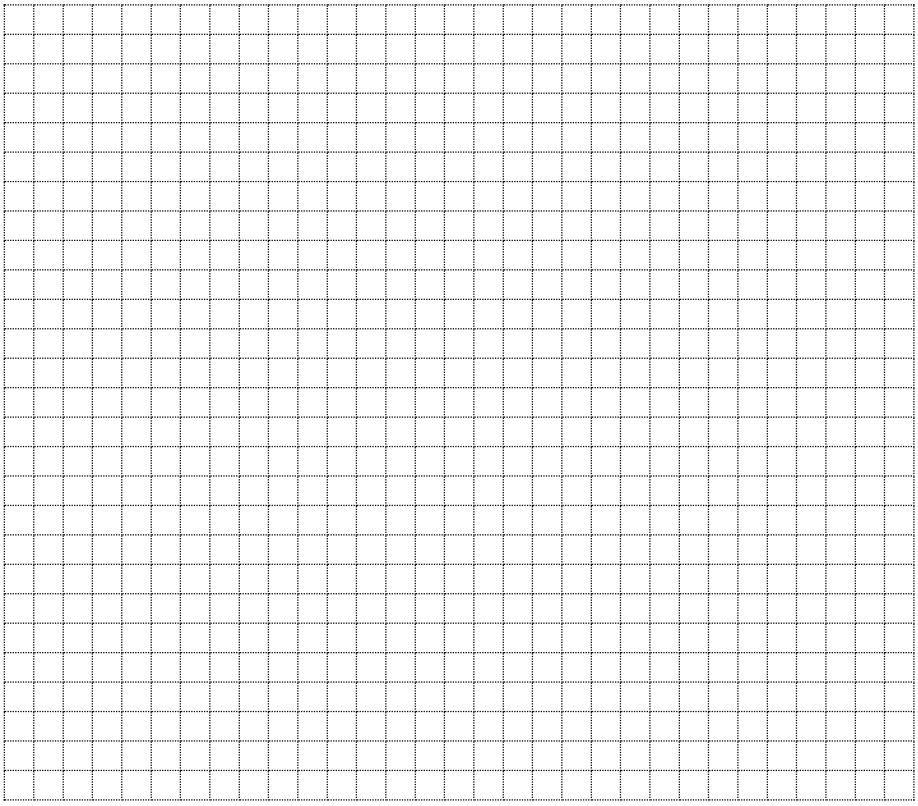
5. Make a list of possible weaknesses and improvements, if any, for this part of the experiment.

Weaknesses in Experiment	Improvement
1.	
2.	
3.	

Part II. Testing the Effect of Temperature on Enzyme Activity

Plot of Rate of Reaction vs. Temperature

1. Using *Excel* or by hand, make a graph of the rate of enzyme activity vs. temperature. Label the graph fully and give it a proper title.



2. Restate the hypothesis you made for this part of the experiment. Does the data support or refute your hypothesis? Summarize what can be concluded from the data. *Quote specific data* to support the conclusions that you make. Your summary should include a discussion on how changing the temperature affects the rate of the decomposition of hydrogen peroxide and why changing the temperature affects the rate of reaction.

3. Use the data collected in this experiment to calculate Q_{10} for catalase: Q_{10} equals the rate of reaction at a given temperature divided by the rate at a temperature 10 ^{o}C lower. How closely does the catalase follow the Q_{10} rule?

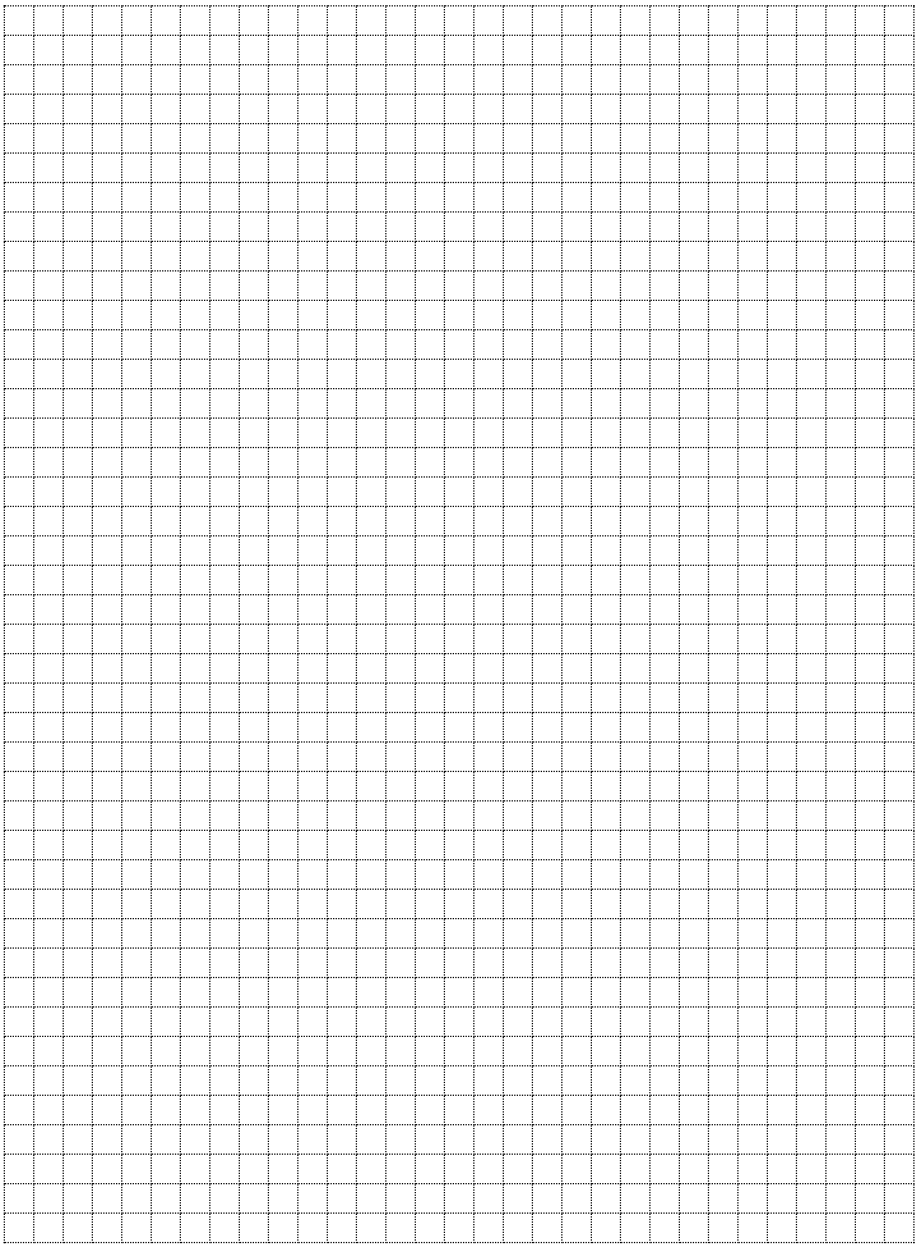
4. Make a list of possible weaknesses and improvements, if any, for this part of the experiment.

Weaknesses in Experiment	Improvement
1.	
2.	
3.	

Part III. Testing the Effect of pH on Enzyme Activity

Plot of Rate of Reaction vs. pH

1. Using *Excel* or by hand, make a graph of Rate vs. pH. Label the graph fully and give it a proper title.



2. Restate the hypothesis you made for this part of the experiment. Does the data support or refute your hypothesis? Summarize what can be concluded from the data. *Quote specific data* to support the conclusions that you make. Your summary should include a discussion on how changing the pH affects the rate of the decomposition of hydrogen peroxide by catalase and why changing the pH affects the reaction rate.

3. Make a list of possible weaknesses and improvements, if any, for this part of the experiment.

Weaknesses in Experiment	Improvement
1.	
2.	
3.	

Application Questions

1. Hot springs at Yellowstone National Park owe their beautiful color to Cyanobacteria (archaebacteria) that live in these very hot (e.g. ~90 ^{o}C) pools of water. Predict, and then draw in the space below a plot of Reaction Rate vs. Temperature for cyanobacteria. Indicate clearly the shape of the curve, and show quantitatively the optimum temperature.

2. Almost all of the food humans eat is digested by more than a dozen different enzymes in the alkaline environment (about pH 8) of the 6-meter long small intestine. Predict, and then draw in the space below a plot of Reaction Rate vs. pH for a typical intestinal enzyme. Indicate clearly the shape of the curve, and show quantitatively the optimum pH.

3. Apply what you have learned in this lab exercise to explain why patients with high fevers feel tired and listless.

4. Some individuals who have been submerged in near freezing water for long periods of time (e.g. 30 minutes) have survived. Apply what you have learned in this lab exercise to explain why this is possible. Note: Submersion in water at room temperature for only a few minutes (e.g. 5 min.) usually results in death. Hint: the cells of all organisms constantly produce toxic waste products as a naturally consequence of their metabolism.

5. Roughly one in four people suffer from lactose intolerance. These individuals have a genetic predisposition that results in a deficiency of lactase, the enzyme that digests lactose, the principle sugar found in milk and milk products). Lactase levels usually start to decline when a child is between 18 and 36 months. Ingestion of milk and milk products may then result in cramps, flatulence, and severe diarrhea. Lactaid tablets, a synthetic version of lactase, can be used by individuals suffering from lactose intolerance to digest the lactose in milk *before* the milk is consumed, thus lowering the production and expulsion of gas, and eliminating diarrhea. **The Question is...** Although Lactaid tablets contain a very *small* amount of lactase, why are they very effective in digesting *large* amounts of lactose?

Extensions / Special Projects

Note: Attempt the following exercises only if your instructor assigns them

1. Organisms live in very different habitats and most have the ability to decompose hydrogen peroxide. Design a series of experiments to investigate how the rate of hydrogen peroxide decomposition varies in different types of organisms. Consider testing a plant, an animal, and a protist.

2. Select 3 different organisms that are capable of decomposing hydrogen peroxide and design a series of experiments to determine for each the optimum conditions for decomposing hydrogen peroxide.

Behind every successful man is a surprised woman.

Maryon Pearson

What are the three words guaranteed to humiliate men everywhere?
"Hold my purse"

Lab 2: Prelab Questions **Nam** _____
Enzymes: Testing Catalase Activity **Group Number** _____ **Date** _____
Biol 211

Instructions: Do the Prelab reading at the beginning of this lab handout *before* attempting to answer the questions that follow! Hand in this assignment just *before* the start of your scheduled lab period.

1. Use your knowledge of chemistry and that of enzyme activity in cells gained by reading the introduction to this lab experiment to explain why the rate of enzyme activity is fastest at the start of an enzyme catalyzed reaction and gradually declines as the reaction proceeds.

2. Under what conditions would the rate of enzyme activity (e.g. catalase's activity) in a cell remain constant and not decline? Explain.

3. Read the procedures for Parts I - III.
   - For each of the three parts of the experiment develop an "If ..., then ..." hypothesis that predicts the results. Record your hypotheses in the appropriate spaces below.
   - Summarize what will be done in each part in the spaces below. To show what is expected, the summary for part I is done for you as an example.

Part I. Testing the Effect of Enzyme Concentration on Enzyme Activity

Hypothesis:

Summary of the Procedure for Part I:

The effect of enzyme concentration on the rate of decomposition of hydrogen peroxide will be investigated by monitoring the pressure of oxygen gas produced with a gas pressure sensor hooked up to a computer. Five test tubes containing aqueous solutions of H_2O_2 (3.0 mL water and 3.0 mL 3.0 % H_2O_2 in each tube) will be prepared. After setting up the apparatus as illustrated in figures 4-6 and (stopper assembly valve open to the atmosphere), add 2 drops of the enzyme suspension to tube #1, stopper immediately, and mix thoroughly. Close the stopper assembly air valve to the atmosphere as in figure 6B, and begin measuring the gas pressure by clicking on the start button. Data will be collected until a linear relationship is observed. The rate of the reaction will then be determined graphically by using computer software as explained by step 12 of the procedure.

The above procedure will be repeated using 5 drops of enzyme suspension in tube 2, 10 drops in tube 3, and 15 drops in tube 4, and with no enzyme in tube 5.

Part II. Testing the Effect of Temperature on Enzyme Activity

Hypothesis:

Summary of the Procedure for Part II:

Part III Testing the Effect of pH on Enzyme Activity

Hypothesis:

Summary of the Procedure for Part III: