ADVANCED PLACEMENT® BIOLOGY

Laboratory 2

Enzyme Catalysis

TEACHER’S MANUAL
LABORATORY 2. ENZYME CATALYSIS

Objectives
In this laboratory, students will
- observe the role of an enzyme (catalase) in the conversion of hydrogen peroxide (H₂O₂) to water and oxygen
- determine the rate of the enzyme-catalyzed reaction

Required Knowledge
Before beginning this laboratory, students should understand
- the general functions and activities of enzymes
- the relationship between the structure and function of enzymes
- the concept of initial reaction rates of enzymes
- how the concept of free energy relates to enzyme activity
- that changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions

Expectations
At the completion of this laboratory, students should be able to
- measure the effects of changes of temperature, pH, enzyme concentration, and substrate concentration on rates of an enzyme-catalyzed reaction in a controlled experiment
- explain how environmental factors affect the rate of enzyme-catalyzed reactions

Time Requirements
All of the exercises in Laboratory 2 can be completed in two 50-minute laboratory periods on two consecutive days. Scheduling the activities in this lab can be a bit complex. Here is an overview of the activities:

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Description</th>
<th>Approximate Time Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>Observe the reaction</td>
<td>25 minutes</td>
</tr>
<tr>
<td>2B</td>
<td>Establish baseline for reaction</td>
<td>25 minutes</td>
</tr>
<tr>
<td>2C</td>
<td>Determine spontaneous rate of decomposition</td>
<td>Day One: 5 minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day Two: 25 minutes</td>
</tr>
<tr>
<td>2D</td>
<td>Determine enzyme-catalyzed rate of reaction</td>
<td>45–50 minutes</td>
</tr>
</tbody>
</table>

Note that times given above encompass lab activity time only. Time needed for constructing graphs, etc. is not included. The key activities are 2B and 2D, and it is best if these two activities are performed on the same day. The baseline amount of H₂O₂ that is determined in 2B is used as a comparison point for the data collected in 2D. Because H₂O₂ spontaneously decomposes over time, if 2B and 2D are not done on the same day, it may introduce an error into the data. Following are some suggestions for scheduling the activities.
On the day before Lab Day 1

- review enzyme structure and function
- introduce the reaction that students will be investigating
- demonstrate the titration procedure

If your class is small enough for students to gather around and observe, consider performing Exercise 2A as a demonstration. Have students record the results of the titration, and set aside 15 mL of H₂O₂ for a student team to use next day (see Student Laboratory, Exercise 2C, Step 1). This would allow you to skip Lab Day 1, below.

Lab Day 1: Students complete 2A and perform the first part of 2C.

Lab Day 2: Assign one student group to complete 2C while other groups are performing 2B. Note that the baseline determined should be approximately the same for all groups. All groups should then complete 2D.

**Preparation**

Photocopy the Student Guide from this manual for your class.

**Titration syringes**
This kit provides materials for performing the titrations with syringes so that you do not have to use burets. Prepare titration syringes by attaching a segment of Tygon tubing to the hub of a 5–6-mL syringe. Prepare one titration syringe per student group. You may use burets if you wish.

**Solutions**
1.0 M H₂SO₄: Supplied ready to use.
2% KMnO₄: Supplied ready to use.
1.5% (0.44 M) H₂O₂: The solution supplied in the kit is 3%. To prepare a 1.5% solution, pour the contents of the bottle into a clean 1-L container. Add 473 mL of distilled water to the container and mix. Store in appropriately labeled, sealed amber bottles (the original bottle may be used as a storage container, but remember to change the label).

**Catalase**
Freeze-dried catalase is supplied with this kit. Only a small amount is required for the exercises, so the container appears nearly empty. The freeze-dried enzyme is stable for 7 to 10 days at room temperature and at least a year frozen. The enzyme is shipped at room temperature, but we recommend that you put it in the freezer when you receive it. Once you add water to reconstitute the enzyme, store the solution in a refrigerator or on ice.

It will retain activity for one week. It is best to add water to the freeze-dried catalase just before beginning the exercises. To reconstitute the enzyme for laboratory exercises, add distilled water up to the shoulder of the bottle (about 120 mL). Shake to dissolve the enzyme. Store in a refrigerator.
Label cups and syringes

At a convenient time prior to Laboratory 2, place the provided labels on cups and syringes, as follows.

*Note: When you label the syringes, do not cover the volume marks.*

<table>
<thead>
<tr>
<th>Number per Group</th>
<th>Item</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10–12-mL syringe</td>
<td>H$_2$SO$_4$</td>
</tr>
<tr>
<td>1</td>
<td>10–12-mL syringe</td>
<td>H$_2$O$_2$</td>
</tr>
<tr>
<td>1</td>
<td>5–6-mL syringe</td>
<td>Transfer</td>
</tr>
<tr>
<td>1</td>
<td>200-mL plastic cup</td>
<td>H$_2$O$_2$</td>
</tr>
<tr>
<td>1</td>
<td>200-mL plastic cup</td>
<td>H$_2$SO$_4$</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Catalase</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>dH$_2$O</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Wash Water</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Waste</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>KMnO$_4$</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Uncatalyzed Decomposition</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>H$_2$O$_2$ Overnight</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>Titration</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>10 sec</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>30 sec</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>60 sec</td>
</tr>
<tr>
<td>1</td>
<td>60 mL plastic cup</td>
<td>120 sec</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>180 sec</td>
</tr>
<tr>
<td>1</td>
<td>60-mL plastic cup</td>
<td>unlabeled</td>
</tr>
</tbody>
</table>
Student Materials and Equipment

Following is a list of the materials needed for one group of students to perform the exercises in this lab. Prepare as many setups as needed for your class. Prepare the setups according to how you schedule the activities. For example, if you follow the schedule suggested in “Time Requirements,” above, on Lab Day 1 students will need all the materials listed for Exercise 2A plus 15 mL of H₂O₂ in a 60-mL cup labeled H₂O₂ Overnight for 2C.

Setup for Each Group

<table>
<thead>
<tr>
<th></th>
<th>Exercise 2A</th>
<th>Exercise 2B</th>
<th>Exercise 2C</th>
<th>Exercise 2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O₂ in 200-mL cup labeled H₂O₂</td>
<td>30 mL</td>
<td>10 mL</td>
<td>80 mL</td>
<td></td>
</tr>
<tr>
<td>200-mL cup labeled H₂SO₄</td>
<td>10 mL</td>
<td>10 mL</td>
<td>80 mL</td>
<td></td>
</tr>
<tr>
<td>catalase in 60-mL cup labeled Catalase</td>
<td>6 mL</td>
<td></td>
<td>8 mL</td>
<td></td>
</tr>
<tr>
<td>KMnO₄ in 60-mL cup labeled KMnO₄</td>
<td>10 mL</td>
<td>10 mL</td>
<td>50 mL</td>
<td></td>
</tr>
<tr>
<td>H₂O₂ in 60-mL cup labeled H₂O₂ Overnight</td>
<td></td>
<td></td>
<td>15 mL</td>
<td></td>
</tr>
<tr>
<td>60-mL cup, unlabeled</td>
<td>1</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>60-mL cup labeled Baseline</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-mL cup labeled Titration</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60-mL cup labeled Uncatalyzed Decomposition</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60-mL cup labeled Wash Water</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60-mL cup labeled Waste</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5 60-mL cups labeled 10 sec, 30 sec, 60 sec, etc.</td>
<td>1 set</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>test tube</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–12-mL syringe labeled H₂O₂</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10–12-mL syringe labeled H₂SO₄</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5–6-mL syringe labeled Transfer</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>titration syringe</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>transfer pipet</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>*glass rod</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*distilled water in 60-mL cup labeled dH₂O</td>
<td>1 mL</td>
<td>1 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*test tube rack</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*test tube holder</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*cube of potato or liver</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*boiling water bath</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*ice bucket and ice</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Items not included in kit.

Students will need graph paper, rulers, and calculators for analyzing their results.

If you wish to demonstrate the titration technique used on Day 2, you will need 10 mL of H₂O₂, 10 mL of H₂SO₄, 5 mL of KMnO₄, and a titration syringe.

At the end of Day 1, have students rinse out all of their containers and syringes; some will be reused on Day 2. Collect everything. The plastic transfer pipets may be discarded or reused.

It is especially important that all equipment used in this exercise be clean, well organized, and labeled. To ensure success of the laboratory exercise, it is important to avoid cross-contamination of the enzyme, substrate, or acid. Caution students to be sure
they are using the correct syringe (“H₂O₂” looks a lot like “H₂SO₄”) and to remember to rinse the Transfer syringe between steps, as called for in the student instructions.

Have students perform the exercises in order. The single transfer pipet should first be used for distilled water, then catalase, to prevent contamination. If you need to change the order of the exercises, make sure that students do not pipet the water for controls (Exercises 2B and 2C) with a pipet they have used for catalase (Exercise 2D).

If you are using burets, clean them so that the KMnO₄ does not bead on the inside. Do not leave KMnO₄ in burets overnight. Since the KMnO₄ is opaque, the readings should be taken from the top of the meniscus.

**Safety Equipment**

Students should wear safety glasses and aprons. Gloves are provided in the kit for two students per group to handle chemicals on Day 2. No gloves are needed in Exercise 2A or for handling H₂O₂.

1. Have students perform the experiment they designed in Question 9, above. The effects of substrate concentration could also be tested.

2. You can demonstrate the protein nature of enzymes by destroying the catalytic properties of a sample of catalase solution with a protease (e.g., trypsin). Compare the activity of the protease-treated catalase with the activity of untreated catalase.

3. Students could prepare catalase directly from beef liver and compare its activity to that of the solution in the kit. To make the comparison, they should calculate the number of units of activity present in their preparation and in the kit solution. One unit of pure catalase decomposes 1 µmole of H₂O₂ per minute at 25°C at pH 7.

Materials required for this procedure are cheesecloth, beef liver, glass rods, and a clean 25-mL test tube for extraction. Prepare the catalase solution as follows:

- Place a piece of fresh liver about 1 cm³ in size in a 25-mL test tube with 10 mL of cold tap water. Macerate using a glass rod for one minute.
- Fill the test tube with about 10 mL of cold water and thoroughly mix the water and the macerated liver. Pour the solution through two layers of cheesecloth into a clean beaker, retaining the liver in the test tube.
- Refill the test tube with 20 mL of cold water and macerate the liver again. Pour this solution through a piece of cheesecloth into the same beaker.

The solution now in the beaker is the stock solution of enzyme. To determine the units of activity per mL of solution, run a 60-second reaction with 10 mL H₂O₂ as in the previous student experiments, stopping the reaction with H₂SO₄ and titrating to determine how much H₂O₂ remains after the titration. Compare this result to a baseline measurement and determine the number of µmoles of H₂O₂ that were decomposed. If the extract is too concentrated, all or most of the H₂O₂ will be decomposed. If this happens, dilute the enzyme solution by various amounts until you find an activity level similar to that of the kit solution. This enzyme stock solution can be tested again or used in other experiments.

**Note:** The presence of proteases in beef liver extracts may create variable results. To minimize variation in results, use the extract immediately after preparation and keep it on ice during the experiment.
LABORATORY 2. ENZYME CATALYSIS

Objectives
In this laboratory, you will
- observe the role of an enzyme (catalase) in conversion of hydrogen peroxide (H₂O₂) to water and oxygen
- determine the rate of the enzyme-catalyzed reaction

Required Knowledge
Before beginning this laboratory, you should understand
- the general functions and activities of enzymes
- the relationship between the structure and function of enzymes
- the concept of initial reaction rates of enzymes
- how the concept of free energy relates to enzyme activity
- that changes in temperature, pH, enzyme concentration, and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions

Expectations
At the completion of this laboratory, you should be able to
- measure the effects of changes of temperature, pH, enzyme concentration, and substrate concentration on rates of an enzyme-catalyzed reaction in a controlled experiment
- explain how environmental factors affect the rate of enzyme-catalyzed reactions

Background
Enzymes are proteins produced by living cells. Enzymes act as catalysts in biochemical reactions. A catalyst speeds up the rate of a chemical reaction and makes it possible for the reaction to occur with a lower initial input of energy. One benefit of enzyme catalysis is that the cell can carry out complex chemical activities at a relatively low temperature.

In biochemical reactions the enzyme, E, combines reversibly with its specific substrate, S, to form a complex, ES. One result of this temporary union is a reduction in the energy required to activate the reaction altering the substrate molecule so that the products, P, of the reaction, are formed. In summary:

E + S ⇌ ES ⇌ E + P

Note that the enzyme is not changed in the reaction and can break down additional substrate molecules. Each enzyme is specific for a particular reaction.

Exercise 2A: Test of Catalase Activity

Introduction
The enzyme you will be working with is catalase. Catalase has four polypeptide chains, each composed of more than 500 amino acids. This enzyme occurs universally in aerobic organisms. One function of catalase within cells is to prevent the accumulation of toxic levels of the hydrogen peroxide (H₂O₂) that is formed as a by-product of metabolic processes. Catalase might also take part in some of the many oxidation reactions going on in all cells.
The primary reaction catalyzed by catalase is the decomposition of \( H_2O_2 \) to form water and oxygen:

\[
2 \text{H}_2\text{O}_2 \Rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \text{ (gas)}
\]

This reaction occurs spontaneously, but not very rapidly. Catalase speeds up the reaction considerably. In this experiment, you will determine a rate for this reaction.

**Procedure**

The rate of a chemical reaction may be studied in a number of ways, including the following:

1. Measuring the rate of disappearance of substrate (in this case, \( H_2O_2 \))
2. Measuring the rate of appearance of product (in this case, \( O_2 \) which is given off as a gas)
3. Measuring the heat released (or used) during the reaction

In this experiment, the disappearance of the substrate, \( H_2O_2 \) will be measured as follows (see Figure 2.2 and Figure 2.3 for illustrations of the procedure and the required apparatus):

1. A purified catalase extract is mixed with substrate (\( H_2O_2 \)) in a beaker. The enzyme catalyzes the conversion of \( H_2O_2 \) to \( H_2O \) and \( O_2 \) (gas).

2. Before all of the \( H_2O_2 \) is converted to \( H_2O \) and \( O_2 \), the reaction is stopped by adding sulfuric acid (\( H_2SO_4 \)). The \( H_2SO_4 \) lowers the pH, denaturing the enzyme and thereby stopping the enzyme’s catalytic activity.

3. After the reaction is stopped, the amount of substrate (\( H_2O_2 \)) remaining in the beaker is measured. To assay this quantity, potassium permanganate is used. Potassium permanganate (\( KMnO_4 \)), in the presence of \( H_2O_2 \) and \( H_2SO_4 \), reacts as follows:

\[
5 \text{H}_2\text{O}_2 + 2 \text{KMnO}_4 + 3 \text{H}_2\text{SO}_4 \rightarrow \text{K}_2\text{SO}_4 + 2 \text{MnSO}_4 + 8 \text{H}_2\text{O} + 5 \text{O}_2
\]

The \( H_2O_2 \) is essential to this reaction. Once all the \( H_2O_2 \) has reacted, additional \( KMnO_4 \) will be in excess and will not be decomposed. The addition of excess \( KMnO_4 \) causes the solution to have a permanent pink or brown color. Therefore, the amount of \( H_2O_2 \) remaining is determined by adding \( KMnO_4 \) until the \( KMnO_4 \) color no longer disappears and the whole mixture remains a faint pink or brown, permanently. Do not add \( KMnO_4 \) after this point. The amount of \( KMnO_4 \) added is a proportional measure of the amount of \( H_2O_2 \) remaining (1 molecule of \( KMnO_4 \) reacts with 2.5 molecules of \( H_2O_2 \)).
1. To observe the reaction being studied, use the labeled syringe to transfer 10 mL of 1.5% (0.44 M H₂O₂) into an unlabeled 60-mL plastic cup. Use a plastic transfer pipet to add 1 mL of the catalase solution and observe what happens. Identify the following components of this reaction:
   a. Enzyme ______________________________________________________
   b. Substrate _____________________________________________________
   c. Product(s) ____________________________________________________

2. Describe a test you could perform on the gas given off to show that it is (or is not) oxygen.

3. To demonstrate the effect of boiling on enzymatic activity, use the same transfer pipet (not the H₂O₂ syringe) to transfer 5 mL of purified catalase extract to a test tube. Set the transfer pipet aside. Place the test tube containing the 5 mL of catalase in a boiling water bath for 5 minutes.

   While the catalase boils, rinse out the unlabeled plastic cup used in Step 1. Transfer 10 mL of 1.5% H₂O₂ into the rinsed clean, unlabeled 60-mL plastic cup. Use a clean plastic transfer pipet to add 1 mL of the boiled catalase solution. How does this reaction compare to the reaction using the unboiled catalase?

4. To demonstrate the presence of catalase in living tissue, cut 1 cm³ of potato or liver and macerate it. Rinse your unlabeled 60-mL plastic cup again, then add 10 mL of fresh 1.5% H₂O₂. Add the potato or liver tissue to the cup. What do you observe? What do you think would happen if you boiled the potato or liver before adding it to the H₂O₂?
Exercises 2B–2D: Notes on Technique

This assay involves performing a titration with KMnO₄. You will use either a buret or a syringe for the procedure. If you are using a syringe, first draw a little air into the syringe, then draw in the KMnO₄ solution. The initial volume reading is where the meniscus of the solution (not the syringe plunger) intersects the scale on the syringe. In order to get good results in all these experiments, you must not cross-contaminate syringes and beakers. Be sure to read the directions carefully and use the appropriately labeled equipment. Take the time to be sure that you have selected the correct instrument before proceeding. For example, using the $\text{H}_2\text{SO}_4$ syringe rather than the $\text{H}_2\text{O}_2$ syringe could affect your results dramatically.

Left alone, $\text{H}_2\text{O}_2$ spontaneously decomposes to water and oxygen at a slow rate. Therefore, a solution that contained 1.5% $\text{H}_2\text{O}_2$ at the time of preparation does not necessarily contain 1.5% $\text{H}_2\text{O}_2$ after storage. In addition, the preparation may not have been perfectly precise. To determine the amount of $\text{H}_2\text{O}_2$ initially present in your nominally 1.5% solution, you need to perform all the steps of the procedure without adding catalase (enzyme) to the reaction mixture. This amount is known as the baseline and is an index of the initial concentration of $\text{H}_2\text{O}_2$ in solution.

**Exercise 2B: The Assay**

**Procedure for Establishing Baseline**

1. Use the 10–12-mL syringe labeled $\text{H}_2\text{O}_2$ to put 10 mL of 1.5% $\text{H}_2\text{O}_2$ in the 60-mL plastic cup labeled *Baseline*.
2. Use the plastic transfer pipet to add 1 mL of distilled H₂O (instead of enzyme solution) from the $\text{dH}_2\text{O}$ cup.
3. Use the 10–12 mL syringe labeled $\text{H}_2\text{SO}_4$ to add 10 mL of 1.0 M $\text{H}_2\text{SO}_4$ from the labeled beaker. Use Extreme Care When Handling Acids. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.
4. Mix well.
5. Use the 5-mL syringe labeled *Transfer* to remove 5 mL of solution from the cup labeled *Baseline* and put it in the cup labeled *Titration*. Rinse the *Transfer* syringe by drawing up some water from the *Wash Water* cup and expelling it into the *Waste* cup.
6. Titrate your 5-mL sample to determine the baseline amount of $\text{H}_2\text{O}_2$.

**Titration Procedure**

1. If you are using a titration syringe (the syringe with the piece of Tygon tubing attached), draw in about 0.2 mL of air, then about 5 mL of KMnO₄ solution and record the initial volume reading. If you are using a buret, fill it and record the initial reading.
2. Use the syringe or buret to add KMnO₄ one drop at a time to the solution in the *Titration* cup until a persistent pink or brown color is obtained. Gently swirl the solution after adding each drop.
3. When you get the persistent pink or brown color, record the final volume of KMnO₄ below. Check to be sure that you understand the calibrations on the syringe or buret.
Syringe Readings | Buret Readings
---|---
Initial reading of syringe _________ mL | Final reading of buret _________ mL
Final reading of syringe _________ mL | Initial reading of buret _________ mL
Baseline (Initial – Final) _________ mL KMnO₄ | Baseline (Final – Initial) _________ mL KMnO₄

Remember, the amount of KMnO₄ used is proportional to the amount of H₂O₂ that was in the solution (1 molecule of KMnO₄ reacts with 2.5 molecules of H₂O₂).

Note: Handle KMnO₄ with care. Avoid contact with skin and eyes.

Exercise 2C: The Uncatalyzed Rate of H₂O₂ Decomposition (The Spontaneous Conversion of H₂O₂ to H₂O + O₂)

On the day before performing this activity, transfer 15 mL of 1.5% H₂O₂ to the 60-mL plastic cup labeled H₂O₂ Overnight. Store it uncovered at room temperature for approximately 24 hours.

1. Use the syringe labeled H₂O₂ to transfer 10 mL of H₂O₂ from the H₂O₂ Overnight cup (from Step 1) to the 60-mL plastic cup labeled Uncatalyzed Decomposition.
2. Use the plastic transfer pipet to add 1 mL of distilled H₂O (instead of enzyme solution) from the dH₂O cup.
3. Use the 10–12-mL syringe labeled H₂SO₄ to add 10 mL of 1.0 M H₂SO₄ from the labeled beaker. Use Extreme Care When Handling Acids. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.
4. Mix well.
5. Use the 5-mL syringe labeled Transfer to remove 5 mL of the reaction mixture and put it in the cup labeled Titration. Rinse the Transfer syringe by drawing up water from the Wash Water cup and expelling it into the Waste cup.
6. Titrate your sample with KMnO₄ as you did in Exercise 2B, Titration Procedure.

Syringe Readings | Buret Readings
---|---
Initial reading of syringe _________ mL | Final reading of buret _________ mL
Final reading of syringe _________ mL | Initial reading of buret _________ mL
Amount used after 24 hours _________ mL KMnO₄

Proportional amount of H₂O₂ spontaneously decomposed (mL baseline–mL 24 hours) _________

What percent of the H₂O₂ spontaneously decomposed in 24 hours?

\[
\frac{([\text{mL baseline} - \text{mL 24 hours}] / \text{mL baseline}) \times 100}{\text{percent}}
\]
Exercise 2D: An Enzyme-Catalyzed Rate of H₂O₂ Decomposition

In this experiment, you will determine the rate at which a 1.5% H₂O₂ solution decomposes when the reaction is catalyzed by catalase. To do this, you should determine how much H₂O₂ has been consumed after 10, 30, 60, 120, and 180 seconds. **Important:** If a day or more has passed since you performed Exercise 2B, you must reestablish the baseline by determining the amount of H₂O₂ present in the 1.5% solution. If this is the case, repeat Exercise 2B and record your new data. Now, measure the decomposition of H₂O₂ by catalase at different time intervals.

**Procedure for a Time-Course Determination**

Set up the 60-mL plastic cups with the labels 10 sec, 30 sec, 60 sec, 120 sec, and 180 sec. Use the 10–12-mL syringe labeled H₂O₂ to add 10 mL of H₂O₂ to each cup. One student should add the reagents while another student keeps the time. Read the directions thoroughly before beginning. For the 10-sec and 30-sec tests, measure the H₂SO₄ and have the syringe ready before beginning the assay.

10 seconds

1. Use a transfer pipet to add 1 mL of catalase extract to the 10 sec cup.
2. Swirl gently for 10 seconds.
3. At 10 seconds, add 10 mL of H₂SO₄ (1.0 M).

30 seconds

1. Use a transfer pipet to add 1 mL of catalase extract to the 30 sec cup.
2. Swirl gently for 30 seconds.
3. At 30 seconds, add 10 mL of H₂SO₄ (1.0 M).

60, 120, and 180 seconds

For each time point, repeat steps 1 through 3, as above, but allow the reactions to proceed for 60, 120, and 180 seconds, respectively.

**Titrations**

Use the rinsed Transfer syringe to remove 5 mL from the 10 sec reaction cup and place it in the plastic cup labeled Titration. Rinse the Transfer syringe by drawing in water from the Wash Water cup and expelling it into the Waste cup. Titrate the sample as you did in Exercise 2B, **Titration Procedure**. Should the end point be overshot, there is sufficient sample remaining to repeat the titration. Record your results in Table 2.1.
Record the baseline value obtained in Exercise 2B in all boxes on line A, above.

*Remember that the baseline tells how much H₂O₂ was in the initial 5-mL sample. The difference between the initial and final readings tells how much H₂O₂ was left after the enzyme-catalyzed reaction. The shorter the reaction time, the more H₂O₂ remained and therefore the more KMnO₄ was used in titrating.

Questions

1. Graph your data below.
   a. The independent variable is ____________________________.
      (Use to label the horizontal x-axis.)
   b. The dependent variable is ____________________________.
      (Use to label the vertical y-axis.)

Table 2.1

<table>
<thead>
<tr>
<th>KMnO₄ (mL)</th>
<th>10</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Baseline*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Initial Reading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Final Reading</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Amount of KMnO₄ Used</td>
<td>B minus C for syringe, C minus B for buret</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Proportional Amount of H₂O₂ Used</td>
<td>A minus D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Questions

1. Graph your data below.
   a. The independent variable is ____________________________.
      (Use to label the horizontal x-axis.)
   b. The dependent variable is ____________________________.
      (Use to label the vertical y-axis.)

Graph 2.1

Title: ____________________________

Label (y-axis):
____________________________________
____________________________________

Label (x-axis): ____________________________
2. Consider the line you have drawn on Graph 2.1. What does this line represent?

3. What does Graph 2.1 tell you about the rate of the reaction over time?

To understand your data, keep in mind that essentially two things must happen for the reaction products to form: the enzyme must form the complex with its substrate \((E + S \Rightarrow ES)\) and then the reaction must occur \((ES \Rightarrow E + P)\). Once the enzyme-substrate complex is formed, the reaction will occur at the same rate as long as temperature and pH are constant. To form the complex, the enzyme and substrate molecules must collide in the right orientation to fit together. The more total collisions between enzyme and substrate that happen per unit time, the more chance of a successful collision in that time interval. Therefore the rate of formation of the ES complex is affected by the concentration of substrate and enzyme in the reaction mixture.

In the first few minutes of an enzymatic reaction, the number of substrate molecules is usually so large compared to the number of enzyme molecules that changing the substrate concentration does not (for a short period at least) affect the number of successful collisions between substrate and enzyme, thus, the enzyme is acting on substrate molecules at a constant rate. The slope of the graph line during this early period is called the initial rate of the reaction. The initial rate of any enzyme-catalyzed reaction is determined by the characteristics of the enzyme molecule. It is always the same for an enzyme and its substrate as long as temperature and pH are constant and the substrate is present in excess. The rate of the reaction, therefore, is the slope of the linear portion of the curve.

In addition to graphing the reaction, you can use the data recorded in Table 2.1 (example given below) to calculate the reaction rate for different time intervals using the following formula:

\[
\text{Reaction Rate} = \frac{E_2 - E_1}{t_2 - t_1}
\]

where

- \(E_1\) = initial amount of \(\text{H}_2\text{O}_2\) decomposed
- \(E_2\) = end amount of \(\text{H}_2\text{O}_2\) decomposed
- \(t_1\) = initial time
- \(t_2\) = end time
Consider the example data above. At 10 seconds, 0.6 mL of \( \text{H}_2\text{O}_2 \) has been decomposed.

Then
\[
E_1 = 0.0 \text{ (no } \text{H}_2\text{O}_2 \text{ has been decomposed at the beginning of the reaction)}
\]
\[
E_2 = 0.6 \text{ mL}
\]
\[
t_1 = 0 \text{ sec}
\]
\[
t_2 = 10 \text{ sec}
\]
\[
\text{Reaction Rate} = \frac{0.6 \text{ mL} - 0.0 \text{ mL}}{10 \text{ sec} - 0 \text{ sec}} = \frac{0.6 \text{ mL}}{10 \text{ sec}} = 0.06 \text{ mL/sec}
\]

4. Use the formula and the data you recorded in Table 2.1 to calculate the reaction rates for each of the time intervals and record the results in Table 2.2.

<table>
<thead>
<tr>
<th>Time Interval (Seconds)</th>
<th>10 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (0–10)</td>
<td></td>
</tr>
<tr>
<td>10–30</td>
<td></td>
</tr>
<tr>
<td>30–60</td>
<td></td>
</tr>
<tr>
<td>60–120</td>
<td></td>
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<tr>
<td>120–180</td>
<td></td>
</tr>
</tbody>
</table>

*Reaction rate (mL \( \text{H}_2\text{O}_2 \)/sec)

5. When is the rate the highest? Why?
6. When is the rate the lowest? For what reasons is the rate low?

7. Based upon facts related to enzyme structure and chemistry, explain the inhibiting effect of sulfuric acid on the function of catalase.

8. Predict the effect of lowering the temperature on the rates of enzyme activity you recorded in Table 2.2. Explain your answer.

9. Design an experiment to test the effect on reaction rate of varying one of the following: pH, temperature, or enzyme concentration. Include materials needed and procedures.