Enzyme Catalysis Lab

Pre-Lab Assignment

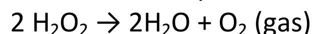
1. Watch the video demonstration for Activity A
2. On a separate sheet of paper, complete the following:
    a. Answer the questions that follows Activity A
    b. Write out the balanced equation for the enzyme catalyzed reaction for hydrogen peroxide
    c. Explain 2 other factors that affect enzyme activity (other than temperature) and what is causing the change in the rate of the reaction.
    d. Draw the general procedure picture
3. In your lab notebooks, set up the following for the lab
    a. Title the lab Enzyme Catalysis Lab using Catalase. Under the title, add the start date of the lab and lab partners (to be filled in on the start date)
    b. Add the lab to your table of contents along with the start date of the lab
    c. Begin with Activity B, write out the information under Baseline Calculations.
    d. For Activity C, write out the information under Uncatalyzed H_2O_2 Decomposition
    e. For Activity D, draw Table 2.1 with title
4. Read through the procedure for Activity B, C, and D. Make sure you are very aware of how to do the lab before the start date.

Background:

In this lab, you will study some characteristics of enzymes action. The specific reaction you will investigate is the decomposition of hydrogen peroxide by the enzyme, catalase. At room temperature, hydrogen peroxide very slowly decomposes into water and oxygen. The addition of catalase lowers the activation energy of the reaction until it proceeds swiftly at room temperature. At the end of the reaction, the catalase is unchanged and is available to catalyze the reaction of more hydrogen peroxide. Catalase, like most enzymes, is a protein. Its ability to form a complex with hydrogen peroxide is based on its molecular shape. Any factor that can alter the shape of a protein molecule can be expected to impact the ability of catalase to facilitate the decomposition of hydrogen peroxide.

Catalase has four peptide chains, each composed of more than 500 amino acids. The enzyme is ubiquitous in aerobic organisms. One function of catalase in cells is to prevent the accumulation of toxic level of hydrogen peroxide formed as a byproduct of metabolic processes. Catalase might also take part in some of the many oxidation reactions that occur in all cells.

The primary reaction catalyzed by catalase is the decomposition of H_2O_2 to form water and oxygen.



$$2\ H_2O_2 \rightarrow 2H_2O + O_2\ (gas)$$

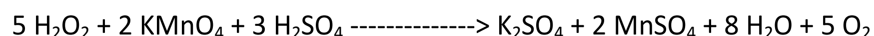
In the absence of catalase, this reaction occurs spontaneously but very slowly. Catalase speeds up the reaction considerably. In this experiment, a rate for this reaction will be determined.

Much can be learned about enzymes by studying the kinetics (particularly the changes in rate) of enzyme-catalyzed reactions. For example, it is possible to measure the amount of product formed, or the amount of substrate used, from the moment the reactants are brought together until the reaction has stopped.

General Procedure

In this experiment the disappearance of the substrate, H_2O_2, is measured as follows.

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, denatures the enzyme, and thereby stops 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 (measure) this quantity, potassium permanganate is used. Potassium permanganate ($KMnO_4$) in the presence of H_2O_2 and H_2SO_4 reacts as follows:



$$5\ H_2O_2 + 2\ KMnO_4 + 3\ H_2SO_4 \text{ --------------> } K_2SO_4 + 2\ MnSO_4 + 8\ H_2O + 5\ O_2$$

Note that H_2O_2 is a reactant in this reaction. Once all the H_2O_2 has reacted, any more $KMnO_4$ added 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 whole mixture stays a faint pink or brown, permanently. Add no more $KMnO_4$ after this point. The amount of $KMnO_4$ added is a proportional measure of the amount of H_2O_2 remaining (2 molecules of $KMnO_4$ reacts with 5 molecules of H_2O_2 as shown in the equation).

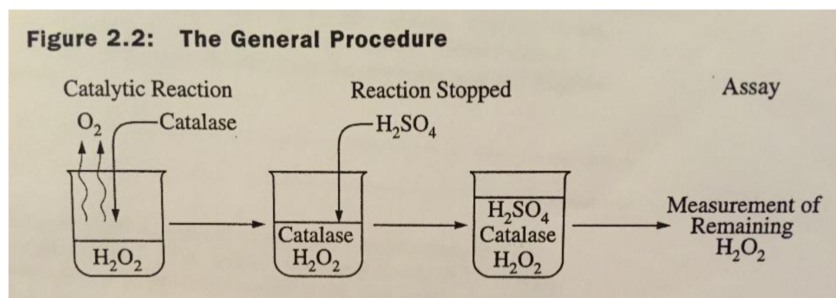


Figure 2.2: The General Procedure

Safety
1. Use extreme care in handling reagents – H_2SO_4
2. Handle $KMnO_4$ with care and avoid contact with skin and eyes

Activity A: Observing the Reaction

Procedure
1. To observe the reaction to be studied, transfer 10 mL of 1.5 % (0.44M) H_2O_2 into a 50 mL glass beaker and add 1 mL of the freshly made catalase solution. The bubbles coming from the reaction mixture are O_2, which results from the breakdown of H_2O_2 by catalase.
   a. What is the enzyme in this reaction?
   b. What is the substrate in this reaction?
   c. What is the product in this reaction?
   d. How could you show that the gas evolved is O_2?
2. To demonstrate the effect of boiling on enzymatic activity, transfer 5 mL of purified catalase extract to a test tube and place it in a boiling water bath for 5 minutes. Transfer 10 mL of 1.5% H_2O_2 into a 50 mL glass beaker and add 1 mL of cooled, boiled catalase solution.
   a. How does the reaction compare to the one using the unboiled catalase? Explain the reason for this difference.

Activity B: The Baseline Assay

To determine the amount of H_2O_2 initially present in a 1.5% solution, one needs to perform all the steps of the procedure without adding catalase to the reaction mixture. This amount is known as the baseline and is an index of the initial concentration of H_2O_2 in solution. In any series of experiments, a baseline should be established first.

Procedure for Establishing Baseline

1. Put 10 mL of 1.5% H_2O_2 into a clean beaker.
2. Add 1 mL of H_2O (instead of enzyme solution).
3. Add 10 mL of H_2SO_4 (1.0 M) Use Extreme care in Handling Acids.
4. Mix well by swirling.
5. Use a new syringe to remove a 5 mL sample of this mixture. Place this 5 mL sample in another beaker for "titration", and assay for the amount of H_2O_2 as follows: Place the beaker containing the sample over white paper. Use a new syringe to add potassium permanganate a drop at a time to the solution until a persistent pink or brown color is obtained. Remember to gently swirl the solution after adding each drop. Record your data below.

Baseline Calculations

Initial reading of syringe ________mL
Final Reading of syringe ________ mL
Baseline (Initial - Final) _________mL $KMnO_4$

The base line assay value should be nearly the same for all groups. Compare your results to another group's before proceeding.

Activity C: The Uncatalyzed Rate of H_2O_2 Decomposition

Background: To determine the rate of spontaneous conversion of H_2O_2 to H_2O and O_2 in an uncatalyzed reaction, put a small quantity of 1.5% H_2O_2 (about 15 mL) in a beaker. Store it uncovered at room temperature for approximately 24 hours. Repeat steps 2-5 from Activity B to determine the proportional amount of H_2O_2 remaining (for ease of calculation assume that 1 mL of $KMnO_4$ used in the titration represents the presence of 1 mL of H_2O_2 in the solution). Record your readings below.

Uncatalyzed H_2O_2 Decomposition

Initial reading of syringe ______________ mL
Final reading of syringe ______________ mL
Amount of $KMnO_4$ titrant ______________ mL

Amount of H_2O_2 spontaneously decomposed (mL baseline – mL $KMnO_4$)
_______________ mL
What percent of the H_2O_2 spontaneously decomposes in 24 hours?
((mL baseline – mL $KMnO_4$) / mL baseline) X 100 _______________ mL

Activity D: An Enzyme-catalyzed Rate of H_2O_2 Decomposition

Background: Having done some initial investigations on the action of catalase, you now need to run a "time-course" determination—which will give you a "snapshot" view of how the reaction proceeds over time. You are going to set up a series of cups, in each of which you will run the same procedure. The only difference will be the amount of time you allow the H_2O_2/catalase reaction to run. You will stop the reaction by adding H_2SO_4. When you have finished timing and stopping all the reactions, you will run a titration to determine how much H_2O_2 remains.

Procedure:

1. Label 7 clean cups as follows: 10 seconds, 30 seconds, 60 seconds, 90 seconds, 120 seconds, 180 seconds, 360 seconds.
2. The larger cups are the storage containers for each of the solutions used in the lab: hydrogen peroxide, H_2SO_4, $KMnO_4$, and a waste cup. There are color coded syringes to match each of the cups.
3. Catalase will be kept in a central location on ice. The 1 mL syringe is reserved for the catalase transfer.
4. Get a stopwatch
5. Put 10 mL of 1.5% H_2O_2 in each of the 7 clean time trial cups.
6. With stopwatch and H_2SO_4 syringe ready, put 1 mL of catalase in the "10 second" cup. Swirl gently and constantly for 10 seconds. At 10 seconds, add 10 mL of H_2SO_4, swirl to mix, and set the cup aside.
7. Get another 1 mL of catalase and set up the stopwatch and H_2SO_4 syringe for the next time trial. Put the catalase in the "30 second" cup. Swirl gently for 30 seconds. At the end of 30 seconds, add 10 mL of H_2SO_4, swirl to mix and set the cup aside.
8. Repeat #7 for each of the remaining cups, varying the amount of time at which you stop the reaction with H_2SO_4.
9. When you have completed the reaction in each of the cups, remove a 5 ml sample from the "10 seconds" cup and determine the amount of H_2O_2 present by running a titration.

Titration: To determine how much hydrogen peroxide (substrate) has been broken down by catalase at varying times, measure the amount of peroxide remaining in each sample. To do so, slowly add $KMnO_4$, which is purple, to the sample. The peroxide in the sample causes the $KMnO_4$ to lose color when the solution is mixed thoroughly. When all the peroxide has reacted with $KMnO_4$, any additional $KMnO_4$ will remain light brown or pinkish even after you swirl the mixture. This is the endpoint. Record the amount of $KMnO_4$ you have used. (The more $KMnO_4$ you use, the more peroxide is left in the sample.) Note: If you overshoot the endpoint, remove another 5 ml sample and start over again! Do not discard any of your solutions until your lab is over! You may need a "do-over".

10. As you determine each value, enter it in Table 2.1.
11. Graph the Amount of H_2O_2 Decomposed by Catalase Over Time. Identify it as Graph 2.1 along with the title and label your axes appropriately.
12. Complete the Analysis of Results.

Table 2.1 “Time-course” Determination of Catalase Activity

time(sec)	10	30	60	90	120	180	360
A. Baseline							
B. Initial Reading $KMnO_4$ (mL)							
C. Final Reading $KMnO_4$ (mL)							
D. Amount of $KMnO_4$ Used (difference between B and C)							
E. Proportional Amount of H_2O_2 Used (A minus D)							

Record the baseline value obtained in Activity B in all boxes on line A, above. *Remember that the baseline tells how much H_2O_2 was in the initial 5-mL sample. The difference between the initial and final readings tells how much H_2O_2 was left after the enzyme-catalyzed reaction. The shorter the reaction time, the more H_2O_2 remained and therefore the more KMnO4 was used in titrating.

Analysis of Results

1. Consider the line you have drawn on **Graph 2.1**. What does this line represent?
2. What does **Graph 2.1** tell you about the rate of the reaction over time?
3. We can calculate the rate of a reaction by measuring, over time, either the disappearance of substrate or the appearance of product.

   $$\text{Reaction rate} = E2 - E1 / t2 - t1$$

   Using the formula above for rate (y/x), determine the initial rate of the reaction and rates between each of the time points in your procedure and record them in **Table 2.2**. Show your math below:

Table 2.2 Rate Determination

Time Intervals (sec)	0 – 10	10 – 30	30 – 60	60 – 90	90 – 120	120 – 180	180 – 360
Rate (mL/sec)							

4. When is the rate the highest? Why is it highest here?
5. When is the rate the lowest? Why is it lowest here?
6. What was the inhibiting effect of sulfuric acid on this reaction? What did the sulfuric acid do to the enzyme and relate it to the enzyme’s structure?
7. What was the purpose of establishing a baseline?
8. Why, when establishing the baseline, are you asked to add 1 mL of water? Why add 10 mL of sulfuric acid to your peroxide samples if there was no catalase to denature?
9. Predict the effect that lowering the temperature would have on the rate of enzyme activity. Explain your prediction.

Designing and Conducting Your Investigation
You now have the basic information and tools needed to explore enzymes in more depth on your own. In this part of the lab, you will do just that. You will have the chance to develop and test your own hypotheses about enzyme activity. To help you get started, read the following questions, and write your answers in your laboratory notebook.

- In the previous lab activity, was the limiting factor of your baseline reaction the enzyme or the substrate? How could you modify the procedure you learned to answer this question?
- What are three or four factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? How would you modify your basic assay to test your hypothesis?

Design and conduct an experiment to investigate an answer(s) to one of the questions above or another question that might have been raised as you conducted the experiment. Remember, the primary objective of the investigation is to explore how biotic and abiotic factors influence the rate of enzymatic reactions. For example, you might test the effect on reaction rate of varying one of the following: pH, salinity, temperature, or enzyme concentration.

Experiments will be conducted time permitting

Analyzing Results
From the data that you collected from your independent investigation, graph the results (or possible results). Based on the graph and your observations, compare the effects of biotic and abiotic environmental factors on the rate(s) of enzymatic reactions and explain any differences. Your report will be presented to the class.

Your write up should include each of the following in paragraph form: question, hypothesis, variables, brief explanation of the set up and measurement of the experiment (not a listed out procedure), potential graph of the data, and final explanation statements about the results.

Color Labels for Cups

Large Cups:

- Orange with matching syringe = H_2O_2
- Red with matching syringe = H_2SO_4
- Dark Green with matching syringe = $KMnO_4$
- Light Green = Waste

Small cups:

- Use regular masking tape to label each time trial cup
- Regular Tan = Water
- Light Green with matching syringe = Assay

Small 1 mL syringe = start with using it for water and then for the enzyme

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