

Investigation 2-13

ENZYME ACTIVITY

How do abiotic or biotic factors influence the rates of enzymatic reactions?

■ BACKGROUND

Enzymes are the catalysts of biological systems. They speed up chemical reactions in biological systems by lowering the activation energy, the energy needed for molecules to begin reacting with each other. Enzymes do this by forming an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. The enzyme's active site is very selective, allowing only certain substances to bind. If the shape of an enzyme is changed in any way, or the protein denatured, then the binding site also changes, thus disrupting enzymatic functions.

Enzymes are fundamental to the survival of any living system and are organized into a number of groups depending on their specific activities. Two common groups are catabolic enzymes (“*cata*” or “*kata*—” from the Greek “to break down”) — for instance, amylase breaks complex starches into simple sugars — and anabolic enzymes (“*a*—” or “*an*—” from the Greek “to build up”). (You may know this second word already from stories about athletes who have been caught using anabolic steroids to build muscle.)

Catalytic enzymes, called proteases, break down proteins and are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin. Bromelain often is an ingredient in commercial meat marinades. Papain is an enzyme that comes from papaya and is used in some teeth whiteners to break down the bacterial film on teeth. People who are lactose intolerant cannot digest milk sugar (lactose); however, they can take supplements containing lactase, the enzyme they are missing. All of these enzymes hydrolyze large, complex molecules into their simpler components; bromelain and papain break proteins down to amino acids, while lactase breaks lactose down to simpler sugars.

Anabolic enzymes are equally vital to all living systems. One example is ATP synthase, the enzyme that stores cellular energy in ATP by combining ADP and phosphate. Another example is rubisco, an enzyme involved in the anabolic reactions of building sugar molecules in the Calvin cycle of photosynthesis.

To begin this investigation, you will focus on the enzyme **catalase** obtained from beef liver extract, one of numerous sources of this enzyme. Catalase is one of several enzymes that break down peroxide, a toxic metabolic waste product of aerobic respiration. Using catalase, you will develop essential skills to examine your own questions about enzyme function.

Later, you will have an opportunity to select an enzyme, research its properties and mode of reaction, and then design an experiment to explore its function. The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the levels of protein structure, energy transfer, abiotic and biotic influences on molecular structure, entropy and enthalpy, and the role of enzymes in maintaining homeostasis.

■ Learning Objectives

- To understand the relationship between enzyme structure and function
- To make some generalizations about enzymes by studying just one enzyme in particular
- To determine which factors can change the rate of an enzyme reaction
- To determine which factors that affect enzyme activity could be biologically important

■ General Safety Precautions

Follow general laboratory safety procedures. Wear proper footwear, safety goggles or glasses, a laboratory coat, and gloves. Use proper titration techniques, and use pipettes, or syringes. Dispose of any broken glass in the proper container. Be particularly cautious when using sulfuric acid to denature your enzyme! Dispose of all chemicals at the direction of your teacher. When you develop your individual investigations you must always consider the toxicity of materials used.

■ Key Vocabulary

Baseline is a universal term for most chemical reactions. In this investigation the term is used to establish a standard for a reaction. Thus, when manipulating components of a reaction (in this case, substrate or enzyme), you have a reference to help understand what occurred in the reaction. The baseline may vary with different scenarios pertinent to the design of the experiment, such as altering the environment in which the reaction occurs. In this scenario, different conditions can be compared, and the effects of changing an environmental variable (e.g., pH) can be determined.

Rate of reaction can be determined with a timed decomposition. At various time intervals the reaction is halted and the amount of substrate remaining is determined by a titration. When you design your own experiment, you can compare the change in a reaction, you can infer increase, decrease, or no change in the rate; this inference is usually called the **relative rate of the reaction**.

■ THE INVESTIGATIONS

■ Getting Started

First, complete this Pearson Lab Bench activity online

http://www.phschool.com/science/biology_place/labbench/lab2/intro.html

And Watch this Video <http://www.bozemanscience.com/science-videos/2012/3/19/ap-biology-lab-2-enzyme-catalysis.html>

■ Part 1: Developing a Method for Measuring Catalase Activity and Determining a Baseline

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Catalase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes. The general reaction can be depicted as follows:



For this investigation the specific reaction is as follows:

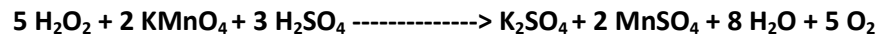


Notice that the catalase is present at the start and end of the reaction. Like all catalysts, enzymes are not consumed by the reactions. To determine the rate of an enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In decomposition reaction of peroxide by catalase (as noted in the above formula), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the *actual* volume of oxygen gas released or by using an indicator. In this experiment, the amount of hydrogen peroxide (substrate) left after the reaction is stopped – *not* converted to product- can be determined by titrating with potassium permanganate (KMnO₄).

General Procedure

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

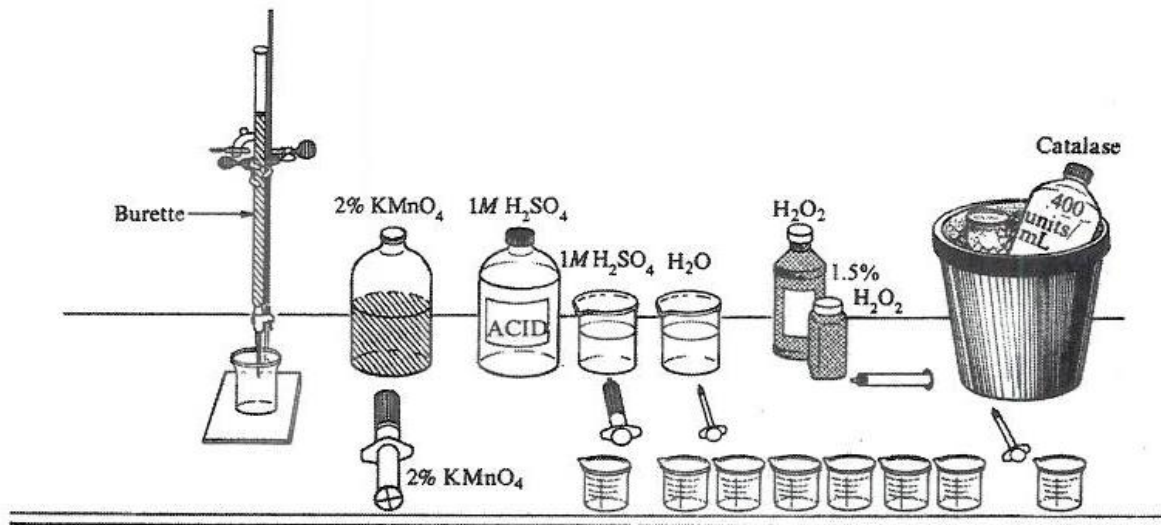
1. A purified catalase extract is mixed with substrate (H_2O_2) in a beaker. The enzyme catalyzes the conversion of H_2O_2 to H_2O and O_2 (gas).
2. Before all the H_2O_2 is converted to H_2O and O_2 , the reaction is stopped by adding sulfuric acid (H_2SO_4). The sulfuric acid lowers the pH, denatures 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 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:



Note that H_2O_2 is a reactant for 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 setup:

Figure 2.3: The Apparatus and Materials



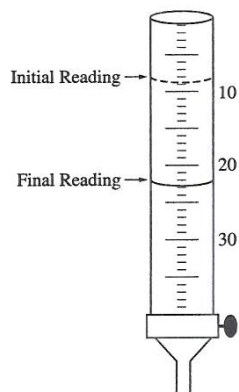
The Baseline Assay

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 burette 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.

Figure 2.4: Proper Reading of a Burette



Baseline calculations

Final Reading of burette _____ mL

Initial reading of burette _____ mL

Baseline (Final -Initial) _____ mL KMnO_4

■ ■ Part 2: 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. You will run your Time Course assays at the same time. This is a lot of work, so you need to know what you are doing and be organized! It is possible to get all of this done, but you must work quickly and efficiently—as a team!

Procedure:

1. Label 7 clean cups as follows: 10 seconds, 30 seconds, 60 seconds, 90 seconds, 120 seconds, 180 seconds, 360 seconds.
2. Fill a beaker with approximately 80 mL of H_2SO_4 . Be careful with H_2SO_4 ! If you spill it on your skin, flush with LOTS of water immediately! (This is a dilute sulfuric acid, but it will still burn if you don't get it off.) Get a 10 mL syringe and reserve it for sulfuric acid. (One group member should be in charge of sulfuric acid.)
3. Get another beaker and put approximately 10 mL of catalase into it. Take it back to your desk and keep it on ice! (This is very important—catalase disintegrates quickly.) Get a 1 mL syringe and reserve it for catalase.
4. Get approximately 80 mL of H_2O_2 in a plastic cup, and then put 10mL in each of the labeled plastic cups.
5. Get a stopwatch and figure out how to use it!
6. 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 (“alphabet time”), and set the cup aside.
7. Put 1 mL of 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 . When you have completed the reaction in each of the cups, remove a 5 ml sample 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”.

9. As you determine each value, enter it in **Table 2.1**.
10. *Graph* the Amount of H_2O_2 Decomposed by Catalase Over Time. Identify it as **Graph 2.1**. Don't forget that you need to title your graph and label your axes appropriately. You can download an Excel version on the website.
11. Complete the **Analysis of Results**. Attach your answers.
12. Be prepared to discuss your results with the class tomorrow (and maybe take a quiz????)

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 KMnO₄ Used (difference between B and C)							
E. Proportional Amount of H₂O₂ Used A minus D							

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.

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?

We can calculate the rate of a reaction by measuring, over time, either the disappearance of substrate or the appearance of product. Reaction rate = $\frac{E_2 - E_1}{t_2 - t_1}$

3. Using the formula above for rate ($\Delta y/\Delta x$), 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. What is meant by V_{\max} in an enzymatic reaction? Mark it on your graph.
5. When is the rate the highest? Why is it highest here?
6. When is the rate the lowest? Why is it lowest here?
7. What was the inhibiting effect of sulfuric acid on this reaction? What did the sulfuric acid do to the enzyme?
8. What was the purpose of establishing a baseline?
9. 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? Please explain!
10. What would lowering the temperature in this reaction do to the rate of enzyme activity?

■ 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 Procedures 1 and 2. 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.

*Submit your proposal to your teacher via email, by _____ (date). Include materials needed (and approximate amounts) and procedures.

■ Analyzing Results

From the data that you collected from your independent investigation, graph the 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 on _____ (date).