

ENZYME ACTIVITY

■ 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 **peroxidase** obtained from a turnip, one of numerous sources of this enzyme. Peroxidase is one of several enzymes that break down **peroxide**, a toxic metabolic waste product of aerobic respiration.

■ 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

* Transitioned from the *AP Biology Lab Manual* (2001)



■ 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 can have more than one applicable definition because this lab has two major options of approach, i.e., using a color palette and/or a spectrophotometer to measure percent of light absorbance. When using a color palette to 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**. When using a spectrophotometer (or other measuring devices) to measure the actual percent change in light absorbance, the rate is usually referred to as **absolute rate of the reaction**. In this case, a specific amount of time can be measured, such as 0.083 absorbance/minute.

■ THE INVESTIGATIONS

■ Getting Started

■ **Procedure 1:** Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase 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 peroxidase 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 a decomposition reaction of peroxide by peroxidase (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, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen to form tetraguaiacol, which is brownish in color. The greater the amount of oxygen gas produced, the darker brown the solution will become.

Qualifying color is a difficult task, but a series of dilutions can be made and then combined on a palette, which can represent the relative changes occurring during the reaction. A color palette/chart ranging from 1 to 10 (Figure 1) is sufficient to compare relative amounts of oxygen produced. Alternatively, the color change can be recorded as a change in absorbency using a variety of available meters, such as a spectrophotometer or a probe system. (Information about the use of spectrophotometers and/or probe systems is found in the Additional Information section of this investigation.)

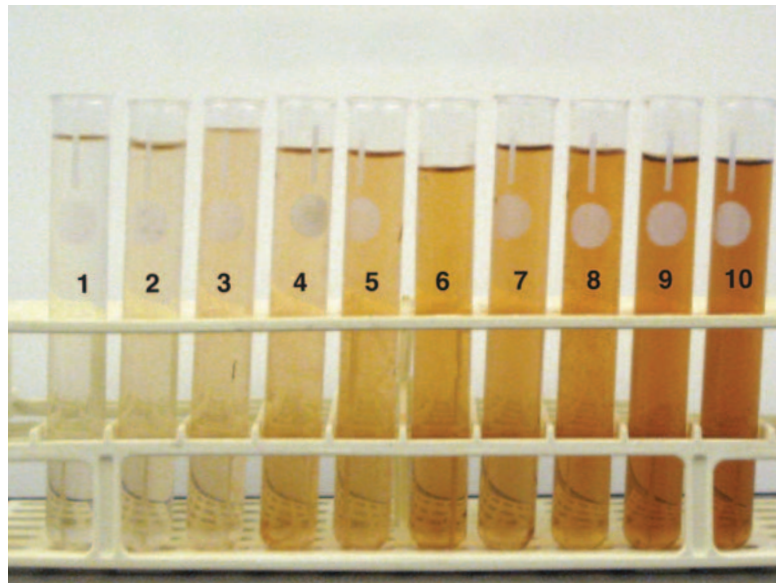


Figure 1. Turnip Peroxidase Color Chart



Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Distilled (deionized) water
- 2 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)

Step 1 Using two 16 x 150 mm test tubes, mark one “**substrate**” and the other tube “**enzyme**.” To the substrate tube, add 7 mL of distilled water, 0.3 mL of 0.1 percent hydrogen peroxide, and 0.2 mL guaiacol for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm® and gently mix.

Step 2 To the enzyme tube, add 6.0 mL of distilled water and 1.5 mL of peroxidase for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm and gently mix.

Step 3 READ STEP 4 BEFORE DOING THIS STEP!!! Combine the contents of the two tubes (substrate and enzyme) in another 16 x 150 ml test tube, cover the tube with Parafilm, invert twice to mix, and place the tube in a test tube rack. Immediately begin timing the reaction.

Step 4 Observe the color change for the next 5 minutes. Rotate the tube before each reading. Record the observed color at 0, 1, 2, 3, 4, and 5 minutes. (A cell phone and/or camera are excellent ways to record color change.)

Step 5 Use the color palette/chart (Figure 1) to help you quantify changes in color over time. Graph your data using Google Sheets.

Consider the following questions before you proceed to the next experiment:

- You measured the color change at different times. Which time will you use for your later test? Why? (The time/color change that you select will serve as your baseline for additional investigations.)
 - Time: _____
- When you use this assay to assess factors that change enzyme activity, which components of the assay will you change? Which will you keep constant?





■ Procedure 2: Determining the Effect of pH on Enzymatic Activity

Numerous variables can be employed to observe the effects on the rate of an enzymatic reaction and possibly the specific fit of the enzyme with the substrate.

- What do you predict will occur if the pH in the reaction changes? How do you justify your prediction?

Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Buffers with range of pH
- Distilled (deionized) water
- 12 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)

Step 1 Using clean 16 x 150 mL test tubes, make six sets of pairs of original **substrate** and **enzyme** tubes for a total of 12 tubes or 6 pairs. This time you will substitute a different pH buffer for the distilled water used in the original enzyme tubes. Prepare the tubes as follows and be sure to label them.

- For each **substrate** tube in a pair, add 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL of guaiacol for a total volume of 7.5 mL.
- For each **enzyme** tube in the pair, add 6.0 mL of a specific pH solution and 1.5 mL of peroxidase for a total volume of 7.5 mL. For example, in the enzyme tube of the first pair, you can substitute 6.0 mL of buffer solution of pH 3 for the distilled water; in the enzyme tube of the second pair, you can substitute 6.0 mL of buffer solution of pH 5 for the distilled water, and so forth.
- Cover each test tube with a piece of Parafilm, and gently mix.

Step 2 Combine the substrate and enzyme tubes for all six pairs (total volume 15.0 mL per pair), cover with Parafilm, gently mix, and place the tubes back in the test tube rack. Immediately begin timing the reactions. (**You may do each separately if necessary.**)

Step 3 Record the observed color for each tube at **0 minutes** and again at the time you chose based on your results in Procedure 1. (**Again, a cell phone and/or camera are excellent ways to record color change.**)



Step 4 Use the palette/color chart (Figure 1) to help you quantify the changes you observe. Graph your data as color intensity versus pH. What conclusions can you draw from your results?

■ 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.

- 1 . Is the peroxidase/hydrogen peroxide enzyme reaction anabolic or catabolic? Explain.
- 2 . What was the purpose of adding the guaiacol to the reaction?
- 3 . What was the purpose of establishing a baseline for the reaction?
- 4 . When examining the rate of the enzyme-substrate reaction, when was the reaction occurring most rapidly? Why? What happened as the reaction continued?
- 5 . Suppose you were investigating a different enzyme-substrate reaction and knew little about the enzyme. Through experimentation you determined that the enzyme functioned most effectively at a pH of 3. What might this suggest about the enzyme?
- 6 . Enzymes have proven to have many uses in commercial applications, including household uses. Give one example of how enzymes might be used in a household application. (Give details on how it's being used, don't just say "as a cleaner".)



Procedure 1: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline Peroxide using the Vernier Colorimeter.

Step 1 Go to the Chrome Web Store and download the Vernier Graphical Analysis App.

Step 2 Once downloaded, close out of your browser and on the lower left of your chromebook screen hit the "launcher" and click "Graphical".

Step 3 Now, use the USB cord attached to your Logger Pro 2 unit and plug this into the chromebook. Give it a couple of minutes to calibrate and it should recognize the colorimeter attached to the Logger Pro 2 unit.

Step 3 Set the wavelength on the colorimeter to 470nm by pressing the left and right arrows.

Step 4 Obtain 3 test 16 x 150mm test tubes and place them in a test tube rack.

Step 5 Using a marker label one of the tubes "S" (for substrate), one of the tubes "E" (for enzyme), and one of the tubes "B" (for blank).

Step 6 The blank will contain everything but the substrate and will be used to zero the colorimeter. Using a 10 ml bulb graduated pipette, add 10 ml of distilled water to the tube labeled "B". Using the same graduated pipette, add 3.3 ml more water to the same tube, for a total volume of 13.3ml water in the tube.

Step 7 Using the "micropipette" at your teacher's desk, add 0.2ml guaiacol to the tube.

Step 8 Using a bulb graduated pipette, add 1.5 ml turnip peroxidase to the tube.

Step 9 Cover the top of the tube with para film, and invert several times to mix.

Step 10 Pour some of the solution from tube "B" into a cuvette.

Step 11 Wipe clean the outside of the cuvette with lens paper.

Step 12 Place the cuvette into the colorimeter and hit "cal" to calibrate the colorimeter.

Step 13 Remove the cuvette containing the blank solution and set aside.

Step 14 Using the 10ml bulb graduated pipette add 7ml of distilled water to the tube labeled "S". Using the same pipette, add 6ml distilled water to the tube labeled "E".

Step 15 Using the same micropipette used to add guaiacol to the blank, add 0.2ml guaiacol to the tube labeled "S".

Step 16 Using the other micropipette, add 0.3ml of hydrogen peroxide to the tube labeled "S".

Step 17 Using the same bulb graduated pipette you used for the turnip peroxidase added to the blank, add 1.5ml of turnip peroxidase to the tube labeled "E".

Step 18 Now, take your two empty cuvettes and fill each about half full. One with liquid from the tube labeled "E" and one with liquid from the tube labeled "S".

Step 18 Now, mix the contents of the enzyme and substrate cuvettes using the funnel provided, place a cap on the cuvette, give it a quick shake to mix, wipe the outside of the cuvette, and place it into the colorimeter. The timer should start timing.

Step 19 Take an absorbance reading immediately, record this in your data table. This will be time 0.

Step 20 Record the absorbance at 2, 3, 4, and 5 minutes. Record these in your data table for the baseline.

Data Table - Determining a Baseline of Absorbance and at different pH

Absorbance / minute						
	0	1	2	3	4	5
Baseline						
pH 3						
pH 5						
pH 6						
pH 7						
pH 8						
pH 10						

Procedure 2: Determining the effect of pH on enzyme activity using the Vernier Colorimeter.

Step 1 Now, obtain 12 test tubes and place them in your test tube rack.

Step 2 Using your marker, label six of the tubes "S" (for substrate) and six of the tubes "E" (for enzyme).

Step 3 Wipe clean your cuvette containing the blank solution in Part 1 and place it into the colorimeter. Hit "cal" to calibrate the colorimeter.

Step 4 Remove the cuvette and set this aside. You will use this blank solution to calibrate the colorimeter between each trial (different pH).

Step 5 Using the 10ml bulb graduated pipette, add 7ml of distilled water to one of the test tubes labeled "S".

Step 6 Using a syringe, add 6 ml of pH buffer 3 to one of the tubes labeled "E".

Step 7 Using the micropipette at your teachers desk, add 0.2ml guaiacol to the tube labeled "S".

Step 8 Using the micropipette at your teacher's desk, add 0.3ml hydrogen peroxide to the tube labeled "S".

Step 9 Using the bulb graduated pipette, add 1.5ml of turnip peroxidase to the tube labeled "E".

Step 10 Now, take your two CLEAN empty cuvettes and fill each about half full. One with liquid from the tube labeled "E" and one with liquid from the tube labeled "S".

Step 11 Now, mix the contents of the enzyme and substrate cuvettes using the funnel provided, place a cap on the cuvette, give it a quick shake to mix, wipe the outside of the cuvette, and place it into the colorimeter. The timer should start timing.

Procedure 2: Determining the effect of pH on enzyme activity using the Vernier Colorimeter. (CONTINUED)

Step 12 Take an absorbance reading immediately, record this in your data table. This will be time 0.

Step 13 Record the absorbance at 2, 3, 4, and 5 minutes. Record these in your data table for the pH being studied.

Step 14 Repeat steps 3-13 for each of the remaining pH buffers (pH 5, pH 6, pH 7, pH 8, pH 10).

***** BE SURE to use a NEW syringe for each buffer and zero the colorimeter with the blank before each remaining trial.**

GRAPH

Use Google Sheets to create a graph which shows the baseline information compared to the results obtained for each pH buffer. Graph time against absorbance with time on the x-axis and absorbance on the y-axis.

ANALYSIS

Answer the analysis items on the previous page. (At the end of the original instructions which used the "color palate".