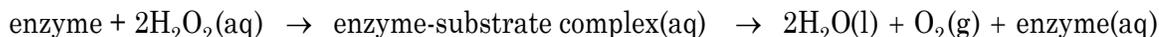


1C. ENZYME ACTIVITY (SPECTROMETER)

Background

Enzymes are *hyper-specific*, that is, usually an enzyme interacts with only one substrate. Like catalysts in other chemical reactions, enzymes are not consumed during the reaction but help turn the substrate into the final product. Notice in the following reaction that the enzyme is present before and after the reaction.



Hydrogen peroxide (H_2O_2) is a byproduct of aerobic respiration in cells and is used in cell signaling and apoptosis. Hydrogen peroxide is highly reactive and can produce free radicals that damage nucleic acids, so cells must carefully regulate its concentration. To remove excess hydrogen peroxide, cells produce an enzyme (such as *catalase* or *peroxidase*) which breaks down H_2O_2 into oxygen (O_2) and water (H_2O), as shown above. This reaction proceeds spontaneously without the enzymes at a very slow rate. This uncatalyzed reaction could serve as the baseline, or control, in an investigation. Peroxidase catalyzes the breakdown of H_2O_2 by assisting the redox reaction between hydrogen peroxide and another compound that acts as a reducing agent, in this case, guaiacol:



Oxygen is not a product because when H_2O_2 is reduced, oxygen is transferred to guaiacol to produce tetraguaiacol. When guaiacol is oxidized to tetraguaiacol, the solution changes color from clear to brownish-yellow. The rate at which this color change occurs indicates the reaction rate.

Driving Question

How does the catalyzed rate of hydrogen peroxide breakdown compare with the spontaneous (uncatalyzed) breakdown rate?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- PASCO Wireless Spectrometer and Spectrometry software
- Cuvettes (2)
- Small test tube with stopper
- Disposable pipets, graduated, 1-mL (5)
- Peroxidase suspension, 0.25 mL
- pH 7 Buffer solution, 3 mL
- Guaiacol solution, 0.75 mL
- 0.1% Hydrogen peroxide (H_2O_2), 1.5 mL
- Kimwipes®

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles and non-latex disposable gloves at all times.
- If using a hot plate, use caution to prevent burns.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

1. Put on your safety goggles and gloves.
2. Designate one clean pipet for each of the three following solutions; do not mix pipets among solutions. Use the pipets to add the amounts shown into a small, clean test tube:

2.0 mL pH 7 buffer

1.0 mL hydrogen peroxide (H_2O_2)

0.50 mL guaiacol

3. Place the stopper on the test tube and invert it 3 times to mix. Use a new, clean pipette to transfer 2.0 mL of the solution from the test tube to the cuvette. Cap the cuvette; this is the “blank” or calibration reference solution.
4. Connect the spectrometer to your device. Open the Spectrometry application and insert the reference cuvette into the spectrometer.

NOTE: Always wipe cuvettes with a lint- and scratch-free wipe before placing into the spectrometer. Orient the cuvette in the spectrometer so the light path is not blocked by labels or cuvette ridges. Never use an acetone-based solvent with the plastic cuvettes.

5. Use the calibration icons at the bottom of the screen to perform a dark calibration and a light calibration.
6. Start collecting data. Scale the graph, and then stop collecting data.
7. To specify the analysis wavelength, move the coordinate tool until the absorbance wavelength reads about 470 nm. Click the check mark to accept the target wavelength.
8. The reference solution is colorless, but when peroxidase is added, the solution will change from colorless to colored. The highest absorbance will be a wavelength around 470 nm. Based on the analysis wavelength, what color might the solution be after the reaction is complete?
9. Remove the reference cuvette from the spectrometer. Select the Time tab in the Spectrometry application.
10. Read through this step (a-d) entirely before proceeding:
 - a. Use designated pipets to add the following to a clean cuvette:

1.0 mL pH 7 buffer
0.5 mL H_2O_2
0.25 mL guaiacol
 - b. Cap the cuvette and invert it 3 times to mix.
 - c. Add 0.25 mL of peroxidase suspension. Quickly cap the cuvette and invert it 3 times to mix.
 - d. Quickly wipe the cuvette and place it in the spectrometer. Start collecting data.
11. After 2 minutes, stop collecting data. Use the linear fit tool to determine the reaction rate in absorbance per second.

12. Why does the addition of the peroxidase suspension cause a color change in the cuvette?
13. Calculate the rate of reaction in absorbance/sec. Copy Table 1 into your lab notebook to record the results.
14. The spontaneous decomposition of hydrogen peroxide is very slow, less than 0.5% per day. When the decomposition was measured in a controlled experiment over several days with a spectrometer using Molecule A as in indicator molecule that undergoes oxidation, the following data was obtained.

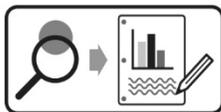
Table 1: Comparison of hydrogen peroxide decomposition rate with and without a catalyst

Indicator Molecule	Spontaneous Rate of Decomposition (absorbance/sec)	Catalyzed Rate of Decomposition (absorbance/sec)	Increase in the Catalyzed Rate (absorbance/sec)
Molecule A	1.8×10^{-7}	0.0031	
Guaiacol	1.8×10^{-7}		

- a. Complete Table 1. How much faster is the catalyzed reaction you observed using guaiacol as the indicator compared to the spontaneous rate of decomposition?
- b. Explain why the reaction is so much faster when an enzyme is present.
15. Is the rate of the reaction constant for the 120 seconds of data collection? Support your answer with evidence.
16. If the reaction continued to run, do you predict the reaction rate to be constant? Explain your thinking.

Design and Conduct an Experiment

Many factors that affect the structure and function of enzymes and the reaction rate of enzyme-catalyzed reactions can be easily manipulated in the lab. Identify one of these factors and design an experiment to determine how that factor affects the rate of an enzyme-catalyzed reaction.



Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan. Then complete the Data Analysis and Synthesis Questions.

Design and Conduct an Experiment: Data Analysis

- From your observations and data,
 - Describe how the independent variable you manipulated affected the hydrogen peroxide breakdown rate. Does the data support your hypothesis? Justify your claim with evidence from your experiment.
 - Based on the evidence you collected, explain why the results occurred.
- Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?
- Identify any new questions that have arisen as a result of your research.

Synthesis Questions

- If you were to double the amount of peroxidase in the initial investigation, how would the reaction rate change? Explain your reasoning.
- Many organisms, such as fungi, animals, and plants, have peroxidase.
 - What does this indicate about the enzyme?
 - Peroxidase is just one of thousands of different enzymes found in plant cells and other organisms. Why do organisms need so many different types of enzymes?
- The graphs below show the relative activity of α -amylase from two different species. Amylase is an enzyme that breaks down complex carbohydrates, like starch, into simple sugars that are used in cell respiration. Figure 1 shows data obtained using α -amylase samples from the bacterium *Bacillus subtilis*, found in the gut of termites across the southern United States.¹

Figure 2 shows data for an α -amylase sample taken from the copepod *Heliodiaptomus viduus*. This organism is found mainly in the Indian Ocean around hot vents. In each case, the enzyme was incubated at a given temperature and then tested for activity at regular intervals.²

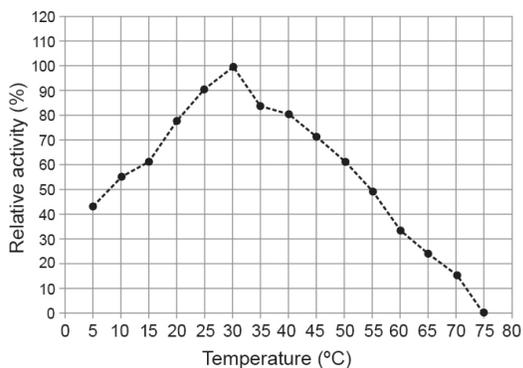


Figure 1. Amylase activity in *Bacillus subtilis*

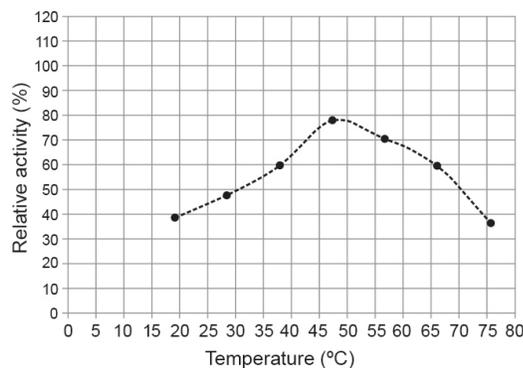


Figure 2. Amylase Activity in *Heliodiaptomus viduus*

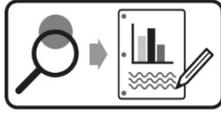
- Discuss how and why temperature affects enzyme activity.
- Explain why the optimal temperature for α -amylase is different for these species.

¹ Femi-Ola, T. O.; Olowe, B. M. Characterization of Alpha Amylase from *Bacillus subtilis* BS5 Isolated from *Ameritermes evuncifer* Silvestri. *Research Journal of Microbiology* 6 (2011): 140–146.

² Dutta, T.K.; Jana, M; Pahari, P. R; Bhattacharya, T. The Effect of Temperature, PH, and Salt on Amylase in *Heliodiaptomus viduus* (Gurney) (Crustacea: Copepoda: Calanoida). *Turkish Journal of Zoology* 30 (2006): 187–195.

Design and Conduct an Experiment Worksheet

Many factors that affect the structure and function of enzymes and the reaction rate of enzyme-catalyzed reactions can be easily manipulated in the lab. Identify one of these factors and design an experiment to determine how that factor affects the rate of an enzyme-catalyzed reaction.



Develop and conduct your experiment using the following guide.

1. Based on your knowledge of enzymes and reactions, what environmental factors (abiotic or biotic) could affect the rates of enzyme-catalyzed reactions?

2. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

3. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

4. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

5. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

6. Write a testable hypothesis (If...then...).

7. What conditions will need to be held constant in the experiment? Quantify these values where possible.

8. How many trials will be run for each experimental group? Justify your choice.

9. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

10. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

11. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

12. Have your teacher approve your answers to these questions and your plan before beginning the experiment.
