1A. ENZYME ACTIVITY (PRESSURE)

Background

Cells have to carry out thousands of chemical reactions very quickly to sustain life. Enzymes are vital to this operation. Enzymes are protein catalysts—they increase the rate of the reaction by lowering the activation energy of the reaction. An enzyme's shape is critical to its ability to catalyze reactions.

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.

$$\text{enzyme(aq)} + 2\text{H}_2\text{O}_2\text{(aq)} \xrightarrow[\text{enzyme complex}]{} 2\text{H}_2\text{O(l)} + \text{O}_2\text{(g)} + \text{enzyme(aq)}$$

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 will serve as the baseline, or control, in the initial investigation.

Driving Question

How does the catalyzed decomposition rate of hydrogen peroxide compare with the spontaneous (uncatalyzed) decomposition 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
- Pressure sensor with connectors and tubing (40-50 cm)
- Sampling bottle or Erlenmeyer flask, 250-mL
- One-hole stopper (to fit sampling bottle or flask)
- Graduated cylinder, 25-mL
- Pipet, 1-mL
- Magnetic stirrer and stirring bar
- Base and support rod
- 3-Finger clamp
- 1.5% Hydrogen peroxide (H_2O_2), 20.0 mL
- Catalase suspension, 2.0 mL

Safety

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

- Wear safety goggles 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.

2. Open the 1A ABI Enzyme-pressure lab file. Connect the pressure sensor to your device.

   NOTE: If the lab file is not available, create a graph of Pressure (kPa) versus Time. Set the sample rate to 15 seconds and set an auto-stop condition for 3 minutes.

3. Put the magnetic stirring bar in the sampling bottle or flask.

4. Set up the equipment as shown in Figure 1.

5. Use a graduated cylinder to transfer 20.0 mL of 1.5% H_2O_2 into the clean 250-mL sample bottle or flask. If the bottle or flask is on a stir plate, set the stir speed to a medium setting.

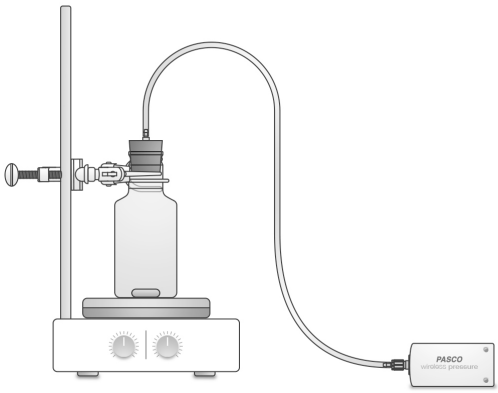


Figure 1: Setup with stirrer

6. Use a pipet to add 2.0 mL of catalase and quickly seal the bottle or flask with the stopper. Begin data collection.

   NOTE: Loosely plug the sample bottle or flask with the stopper. Keep the mixture stirring continuously on a medium setting or swirl the bottle or flask gently by hand, making sure the solution does not spill inside the tubing.

7. Why does the addition of the yeast suspension cause a pressure change inside the sampling bottle or flask?

8. Copy Table 1 into your lab notebook to record the results.

9. When data collection has stopped, calculate the rate of the reaction in kPa/min and record it in your copy of Table 1.

10. The spontaneous decomposition of hydrogen peroxide is very slow, less than 0.5%/day. When the decomposition was measured in a controlled experiment over several days with the pressure and oxygen sensors, the following data was obtained.

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

Sensor	Spontaneous Rate of Decomposition	Catalyzed Rate of Decomposition	Increase in the Catalyzed Rate
Pressure sensor	4.26×10^{-5} kPa/min	RECORD ANSWERS & DATA IN YOUR	
Oxygen sensor	0.33 ppm/min	6,524 ppm/min	NOTEBOOK.

   a. Complete Table 1. How much faster is the catalyzed reaction you observed compared to the spontaneous rate of decomposition?

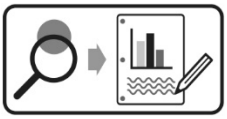
   b. Explain why the reaction is so much faster when an enzyme is present.

11. Is the rate of the reaction constant for the 180 seconds of data collection? Support your answer with evidence.

12. 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

1. From your observations and data,

   a. Describe how the independent variable you manipulated affected the rate of decomposition of hydrogen peroxide. Does the data support your hypothesis? Justify your claim with evidence from your experiment.

   b. Based on the evidence you collected, explain why the results occurred.

2. 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?

3. Identify any new questions that have arisen as a result of your research.

Synthesis Questions

1. If you were to double the amount of catalase in the initial investigation, how would the reaction rate change? Explain your reasoning.

2. Many organisms, such as fungi, animals, and plants, have catalase.

   a) What does this indicate about the enzyme?

   b) Catalase is just one of thousands of different enzymes found in yeast cells and other organisms. Why do organisms need so many different types of enzymes?

3. 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]

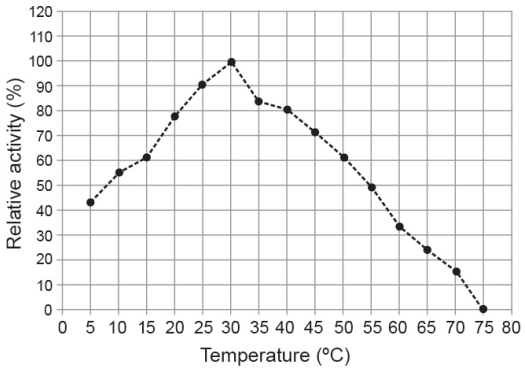


Figure 1. Amlyase activity in *Bacillus subtilis*

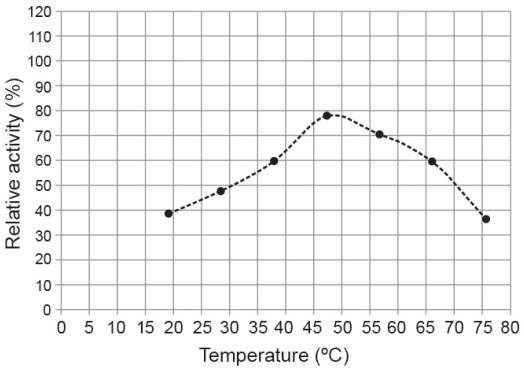


Figure 2. Amylase Activity in *Heliodiaptomus viduus*

   a. Discuss how and why temperature affects enzyme activity.

   b. Explain why the optimal temperature for α-amylase is different for these species.

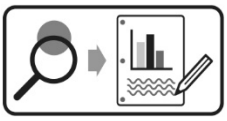
[1] Femi-Ola, T. O.; Olowe, B. M. Characterization of Alpha Amylase from *Bacillus subtilis* BS5 Isolated from *Ameritermes evuncifer* Silvestri. *Research Journal of Microbiology* 6 (2011): 140–146.

[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.

NAME PERIOD DATE

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.

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

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

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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.