

MiSP Enzyme Action Worksheet #1 L3

Name _____

Date _____

TEMPERATURE AND ENZYME ACTIVITY

Introduction:

Hydrogen peroxide (H_2O_2) is a poisonous substance that can be made in a living thing. It can damage cells if it is not removed. Catalase is an enzyme that speeds up the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen gas (O_2).

Hydrogen peroxide with catalase-----> water + oxygen

REMEMBER: CATALASE is an enzyme. An enzyme is an organic CATALYST that increases the rate of a reaction without being used up in the process. Certain plants and animal organs contain high concentrations of catalase. Potatoes and liver are two commonly used sources of catalase. Your teacher has prepared an **extract** of potato by chopping it up into tiny pieces, mixing it with cold water, and removing large chunks. The potato extract contains 100 units of catalase/ml.

Safety:

- YOU WILL BE WORKING WITH HOT WATER.
- WEAR GOGGLES AT ALL TIMES!!!
- Use caution when handling hydrogen peroxide.

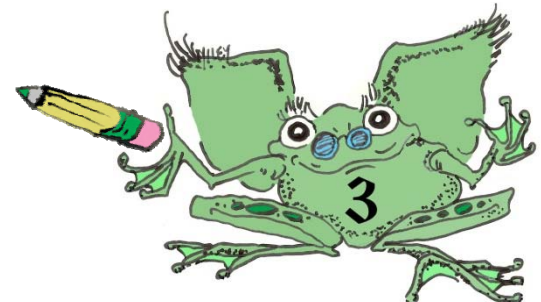
Problem:

- How does temperature affect the rate of enzyme action?
- **How the lab works:**

A filter paper disk will be coated with the potato extract (enzyme source) and then dropped into a vial of the substrate (hydrogen peroxide). As the enzyme breaks down the hydrogen peroxide into water and oxygen gas, the bubbles of oxygen will collect underneath the filter and make it rise to the surface of the hydrogen peroxide. The time it takes for the filter to rise is an indication of the rate of enzyme activity. The LONGER (more seconds) the disk takes to rise to the surface, the SLOWER the reaction. The SHORTER (the fewer seconds) the disk takes to rise to the surface, the FASTER the reaction.

Materials:

- catalase — extracted from potatoes
- 3% hydrogen peroxide
- Forceps
- filter paper disks (holes punched out of filter paper)
- water
- ice



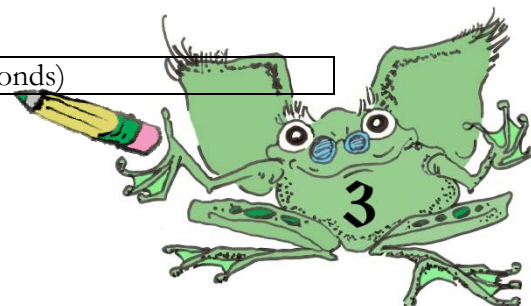
- water baths — ice cold, room temperature, warm (approximately 37°C), and boiling
- vials, little beakers, and/or test tubes
- test tube clamp
- test tube rack if test tubes are being used
- marking pencils
- stopwatch or timer
- 5 ml or 10 ml graduated cylinder (or pipette)
- 50 ml or 100 ml graduated cylinder

Procedure:

- ___ 1. Your teacher will set up water baths and tell you the temperatures. Record the temperatures in your data chart: ice cold, room temperature, warm (approximately 37°C), and boiling.
- ___ 2. Place 5.0 ml of potato extract containing catalase at 100 units/ml in each of 4 test tubes. Label the test tubes with your name and the water bath temperature. Place 1 test tube in each of the water baths.
- ___ 3. Place 5 ml (or a different volume as directed by your teacher) of 3% H₂O₂ in each of 4 vials or 4 additional test tubes. Label. Place 1 vial or test tube in each of the water baths.
- ___ 4. Allow the catalase and the substrate to incubate at each temperature for about 5 minutes. After 5 minutes, test the reaction time at each temperature by dipping a filter paper disk into potato extract (enzyme) at that temperature, draining it, and then dropping it into substrate at the same temp. Time how long it takes the filter to rise to the surface at each temperature. Record your results in the data chart. If the reaction does not occur after 3 minutes, mark the chart for that temperature “n.r.” (no reaction).

Record your data here:

Water Temperature	Reaction Time (seconds)
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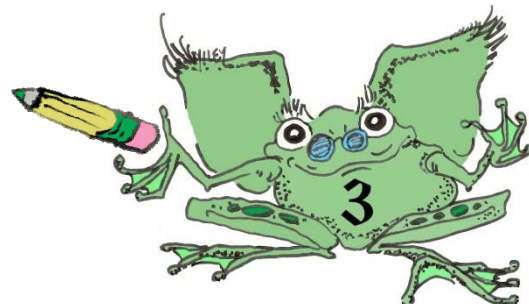


Cold: ____ °C	
Room Temperature: ____ °C	
Warm: ____ °C	
Boiling: ____ °C	

Graph your data:

Graph the data on the next page.

- Label the x -axis.
- Label the y -axis.
- If there is no observable reaction, that data point (temperature) cannot be graphed.
- Draw a best-fit line from the cold ordered pairs to the warm ordered pairs.



Discussion Questions:

1. Which temperature had the fastest reaction (the paper disk came to the top in the shortest time)? Which temperature had the slowest reaction? Was there a temperature at which there was no reaction?

Fastest: _____

Slowest: _____

No reaction: _____

2. Generally, when temperature increases the rate of a reaction increases. Is that true for all temperatures in this experiment? Explain.

- WEAR GOGGLES AT ALL TIMES!!!
- Use caution when handling hydrogen peroxide.

3. Explain the results with the substrate and the enzyme kept in the boiling water bath. (Hint: An enzyme is a protein. The white of an egg is protein. What happens to an egg when it is put in boiling water?)

4. Use the graph to predict the number of seconds for the enzyme-coated disk to rise to the top of the vial or test tube at

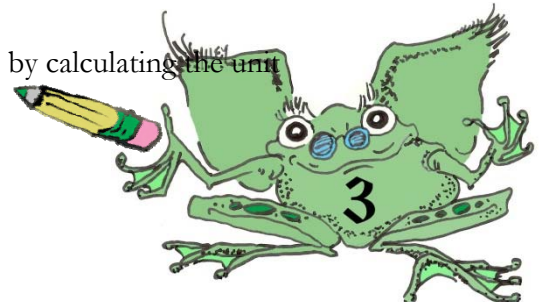
- 10°C _____
- 25°C _____

5. Review your data and write a conclusion statement by completing this sentence (*remember that shorter time indicates faster rate of reaction*):

As the temperature of an enzyme reaction increases, the rate of the reaction

_____.

6. Determine the change in reaction time as temperature increased by calculating the unit rate of change (slope) of the best-fit line.



$$\text{Unit Rate of Change} = \frac{\Delta \text{ Reaction Time (seconds)}}{\Delta \text{ Temperature } ^\circ\text{C}}$$

(When using a best-fit line, the ordered pairs to determine unit rate of change [slope] must be from the best-fit line, not from your data chart.)

$$\text{Unit Rate of Change} = \frac{\Delta \text{ Reaction Time (seconds)}}{\Delta \text{ Temperature } ^\circ\text{C}} = \frac{\Delta y}{\Delta x} = \frac{(y_2 - y_1)}{(x_2 - x_1)}$$

Ordered Pair used for calculation (x_1, y_1) (x_2, y_2)	Δ Reaction Time (seconds) Δy	Δ Temperature $^\circ\text{C}$ Δx	Unit Rate of Change (slope) $\Delta y / \Delta x$

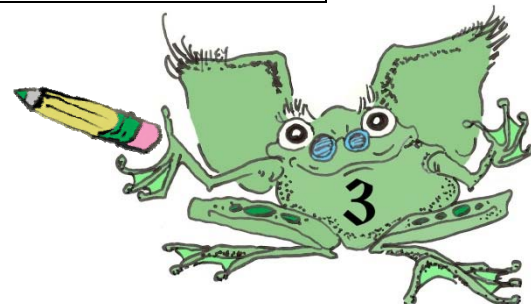
7. What is the sign (-/negative or +/positive) of the unit rate of change? What does that sign tell you about the changes in reaction time (seconds) as the temperature increases?

8. Find the y -intercept for the best-fit line on the graph. Use the equation for a line to calculate the y -intercept. Use the best-fit line you used in #6. The equation for a line is

$$y = mx + b$$

where m is the unit rate of change (slope) and
 b is the y -intercept

Y-Intercept



$$m =$$

Ordered pair $(x,y) = (\text{---} , \text{---})$

$$y = mx + b$$

Solve for b :

9. Based on the unit rate of change (slope) that you calculated above and the y -intercept, write an equation for the best-fit line on the graph. Remember that the equation for a line is $y = mx + b$ and m is the unit rate of change (slope) and b is the y -intercept.

Equation – Temperature and Enzyme Activity Best-Fit line

10. Using the equation above, calculate the reaction time (in seconds) for the indicated temperature.

$$x = 8^{\circ}\text{C}$$

$$y = \text{_____} \text{ seconds}$$

$$x = 31^{\circ}\text{C}$$

$$y = \text{_____} \text{ seconds}$$

