MiSP Enzyme Action Worksheet #2 L2

Name _____ Date _____

SUBSTRATE CONCENTRATION 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:

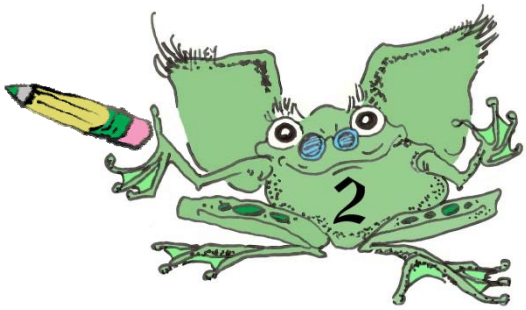
- WEAR GOGGLES AT ALL TIMES!!!
- Use caution when handling hydrogen peroxide.

Problem:

- How does the concentration of the substrate (hydrogen peroxide) affect the rate of enzyme action? The concentration of the enzyme will remain the same.
- ***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
- water
- vials, little beakers, and/or test tubes
- marking pencils



- stopwatch or timer
- 5 ml or 10 ml graduated cylinder (or pipette)
- 50 ml or 100 ml graduated cylinder

Procedure:

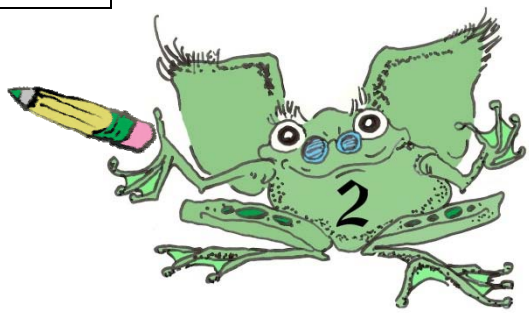
- ___ 1. Obtain 1 vial, medicine cup, or small beaker of catalase at 100 units/ml.
- ___ 2. Dilute the substrate (hydrogen peroxide) as described below. Each dilution should be made in a separate labeled vial or test tube. Label each container. (Volumes of H_2O_2 may be changed by your teacher.)
  - 3.0% H_2O_2: 20 ml 3% H_2O_2
  - 1.5% H_2O_2: 10 ml 3% H_2O_2 + 10 ml distilled water
  - 0.75% H_2O_2: 5 ml 3% H_2O_2 + 15 ml distilled water
  - 0.38% H_2O_2: 2.5 ml 3% H_2O_2 + 17.5 ml distilled water
  - 0.0% H_2O_2 : 20 ml distilled water
- ___ 3. Use forceps to dip a filter disk into the catalase, drain on a paper towel, and then drop the filter into the 3% H_2O_2. Time how long it takes the filter to rise to the top. This is the reaction time. Repeat this procedure for each of the substrate dilutions. Record your results in the data chart. If the reaction does not occur after 3 minutes, mark the chart for that concentration "n.r." (no reaction).

Record your data here:

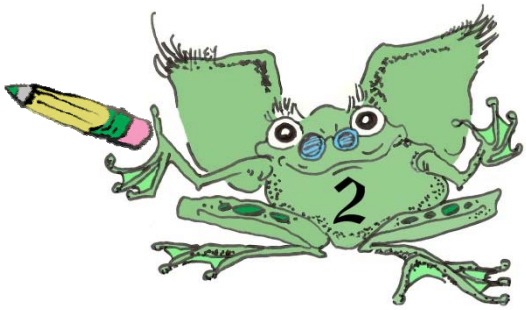
Substrate (Hydrogen Peroxide) Concentration	Reaction Time (seconds)
0%	
.38%	
.75%	
1.5%	
3%	

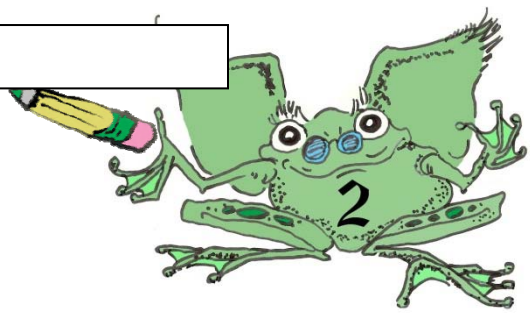
Graph your data:

Graph the data on the next page.



- Label the *x*-axis.
- Label the *y*-axis.
- If there is no observable reaction, that data point (concentration) cannot be graphed.
- Draw a best-fit line from .38% to 3% ordered pairs.

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Discussion Questions:

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

Fastest: _____

Slowest: _____

No reaction: _____

2. Generally, when the concentration of a substance, like hydrogen peroxide, in a chemical reaction is increased, the reaction rate increases (time for the reaction decreases). Was that true in this experiment? Explain.

_____

_____

3. Explain the result when the substrate (hydrogen peroxide) concentration was 0%. (Hint: What would happen to a factory that makes paper if there were no raw materials?)

_____

_____

_____

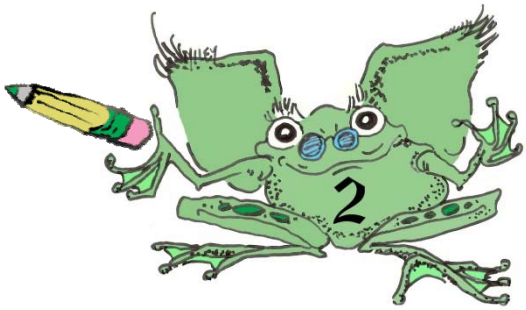
4. Use the graph to predict the number of seconds for the enzyme-coated disk to rise to the top of a vial or test tube filled with the following solutions:

- 1% hydrogen peroxide _____
- 6% hydrogen peroxide _____

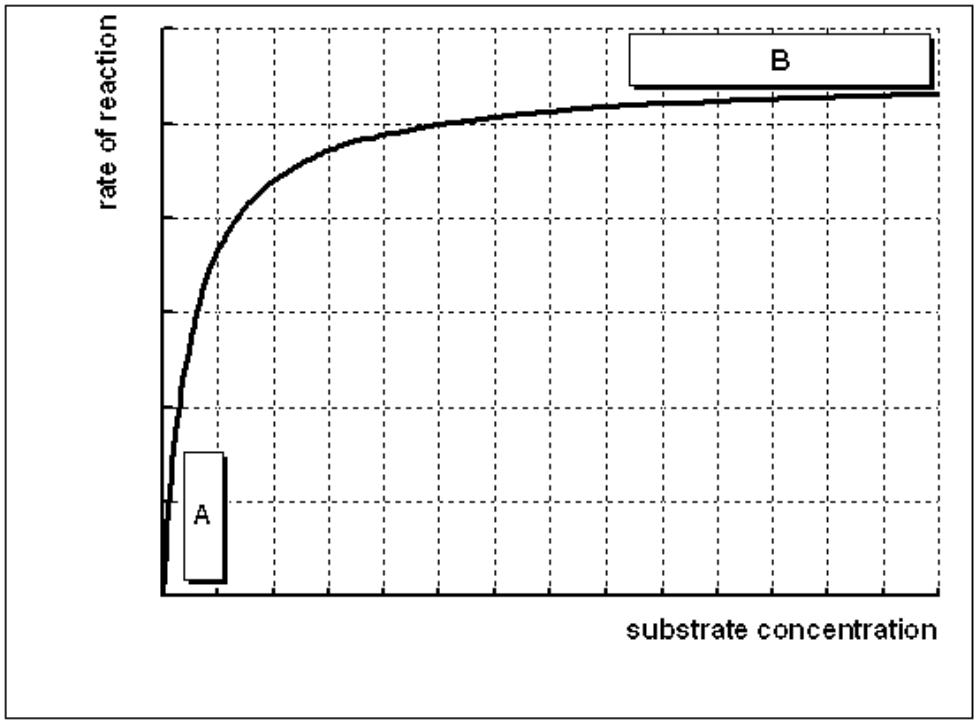
5. Review your data and write a conclusion statement by completing this sentence (remember that shorter time indicates faster rate of reaction):

As the concentration of the substrate in an enzyme reaction increases, the rate of the

reaction _____.



6. The graph of enzyme reaction rates (not reaction time as depicted in this lab's graph) with increasing substrate concentration (enzyme concentration stays the same) looks like this:

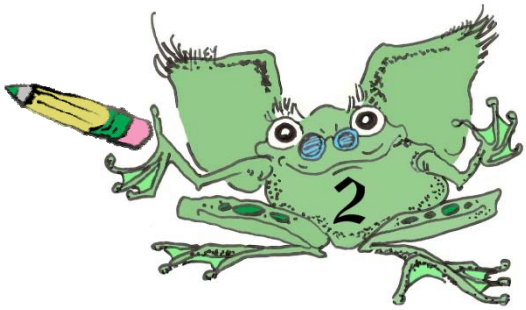


Why does the graph level off at higher substrate concentrations?

_____

_____

_____



7. Calculate the change in reaction time as concentration increased by calculating the unit rate of change (slope) of the best-fit line. *(If you use 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.)*

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

Ordered Pair used for calculation (x_1, y_1) (x_2, y_2)	Δ Reaction Time (seconds) Δy	Δ Concentration (%) Δx	**Unit Rate of Change (slope)** $\Delta y / \Delta x$

8. 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 concentration increases?