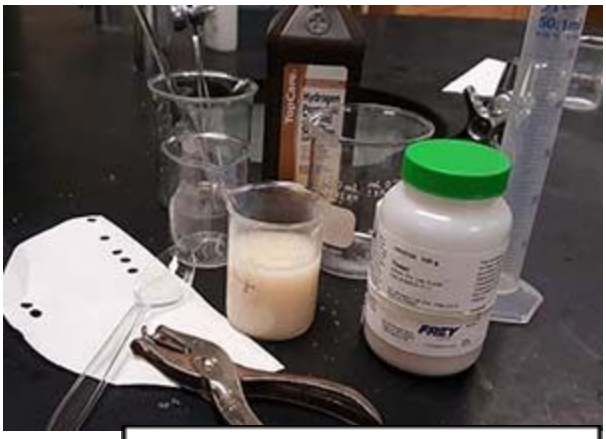
Name:_____Date:_____

Investigation: How Does Concentration Affect the Reaction Rates of Enzymes

Objectives:

- to observe the catalytic action of yeast catalase on hydrogen peroxide
- to determine the effect of substrate and enzyme concentration catalase activity



Materials

Hydrogen Peroxide (3%)
4 beakers or cups for H_2O_2 Dilutions
5 small cups for yeast dilutions
Graduated cylinders
Filter paper and Hole punch
Forceps
Stopwatch or timer
Active dry yeast

Background information:

Hydrogen peroxide (H_2O_2) is a common but poisonous by-product of cellular metabolism, but H_2O_2 does not accumulate in cells because it is decomposed into water and oxygen gas. The decomposition of the hydrogen peroxide is facilitated by catalase, an enzyme present in most cells.

The reaction is: $\mathbf{2H_2O_2 \rightarrow 2H_2O + O_2}$

One molecule of catalase can catalyze the decomposition of approximately 4 x 10^7 molecules H_2O_2 per second! Catalase is stored in special vesicles of the cell called peroxisomes.

In this lab activity, you will be using yeast catalase to observe how increasing and decreasing the concentration of the enzyme and substrate can affect the reaction rate.

Create your stock catalase solution

1. Dissolve 1 tsp (2-4 grams) of yeast in 200 ml of warm water. **This solution may already be made for you.*
2. Mix well and let sit for about 3 minutes
3. Stir gently to keep solution and yeast mixed.

Observation of Catalase Activity

1. Pour 80 ml of H_2O_2 into a small beaker.
2. Cut a filter paper disk using a hole punch
3. Use forceps to soak a the punched disk in your stock catalase
4. Place the disk into a beaker with H_2O_2.

Observe what happens to the disks when dropped into peroxide.
If nothing happens you may need to troubleshoot your experiment. Try stirring your catalase solution or dipping the disk in the H_2O_2 to break the surface tension. If you are still not seeing anything happen, consult your instructor.

4. Perform this procedure again and record the time it takes for the disk to drop and then raise to the surface. Perform multiple trials to perfect your technique. Convert all readings to seconds to take an average. **Place your data in the first column of the data table.**

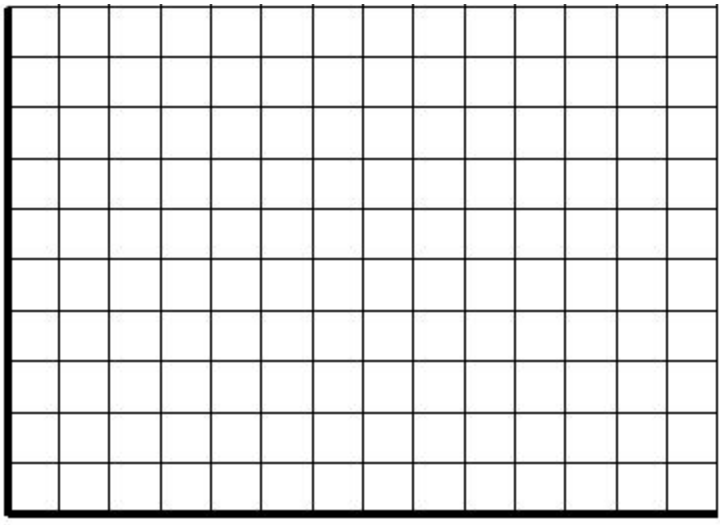
Effect of Substrate Concentration

The H_2O_2 started at 3%. You will now dilute the peroxide in order to change its concentration.

1. Place 40 ml of H_2O_2 into a new beaker. Add 40 ml of water. H_2O_2 concentration = 1.5%
2. Place 20 ml of H_2O_2 into a new beaker. Add 60 ml of water. H_2O_2 concentration = .75%
3. Place 10 ml of H_2O_2 into a new beaker. Add 70 ml of water. H_2O_2 concentration = .375%
4. Perform the floating disk procedure for each concentration. *You can do multiple trials at the same time.*

	3% H_2O_2	1.5% H_2O_2	0.75% H_2O_2	0.375% H_2O_2
Trial 1				
Trial 2				
Trial 3				
Average				

5. Create a graph that compares the averages for each concentration.



6. Based on your data, how does the <u>concentration of the substrate</u> affect the enzyme reaction rate? Suggest a REASON for these results.

Effect of Enzyme Concentration

Your stock solution of catalase is your 100% solution. Create diluted solutions according to the ratios below and place each in small cups. These cups will be used to dip your filter paper disks.

1. 100% = 20 ml of catalase + 0 ml of water
2. 80 % = 16 ml of catalase + 4 ml of water
3. 60% = 12 ml of catalase + 8 ml of water
4. 40% = 8 ml of catalase + 12 ml of water
5. 20% = 4 ml of catalase + 16 ml of water.

6. Perform the floating disk procedure for each concentration. (We will not be taking averages this time.)

	100% catalase	80% catalase	60% catalase	40% catalase	20% catalase
Time					

7. Use your data to make a **CLAIM** that answers the question: How does the concentration of the enzyme affect the reaction rate.

8. Provide **EVIDENCE** for this claim by briefly summarizing your data or observations.

9. Consider how enzymes and substrates interact with each other. Suggest a **REASON** for your claim. This is where you consider what scientists understand about enzymes. Your reasoning can include sketches that show your understanding of enzymes and substrates.

Synthesis

10. A competitive inhibitor attaches to the active sites of enzymes. Discuss how this would change the rate of the reaction rate of catalase. . Create a model (drawing) to support your claim.

11. Describe how you could use the same technique to test the effects of temperature on reaction rates.

12. The beginning of this lab lists two objectives. Summarize what you have learned in this lab to accomplish those two objectives.

Teacher notes: To save time, I make catalase solution about 20 minutes before the lab, this also gives the yeast time to become active. Beakers work best for the hydrogen peroxide because you can see the disks on the bottom (clear plastic cups can substitute). Dixie cups or medicine cups work for peroxide dilutions.

Students observe that not all their tests are the same. I usually discuss why biological experiments are messy and inconsistent. Some note that the color of the yeast solution varies from top to bottom and that it might matter how far you dip your disks in and how long you keep them there.