Name _____

Catalase Enzyme "DOT" Lab

AKA "Sinkers" or "Floaters"

Introduction

Enzymes are proteins that SPEED UP chemical reactions in living cells. They are biological catalysts, which speed up chemical reactions by **decreasing** the activation energy need to begin a reaction. In this lab, you will learn about enzyme characteristics and explore several **factors that may affect enzyme FUNCTION**. The chemical reaction we will study in this lab involves the hydrogen peroxide (H_2O_2), which naturally decomposes very slowly into water and oxygen gas:

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

"SLOW Process"

The reaction happens very slowly, but over a number of years a bottle of peroxide will convert almost entirely into water.

The enzyme we will study in this lab is called catalase and it dramatically speeds up the breakdown of hydrogen peroxide. Catalase exists in the cells of any organism that breaths oxygen and gets energy for cells through aerobic respiration. Thus, catalase is a common enzyme found in humans to reduce toxic levels of H_2O_2 that accumulate as a metabolic byproduct, or waste as our cells generate energy. In other words, any cell without catalase enzymes would soon die from the toxic buildup of H_2O_2. The rate of the catalyzed breakdown of hydrogen peroxide can be measured by the rate of O_2 production. In this investigation the speed with which O_2 bubbles cause a paper disk to rise indicates the relative speed of the reaction.

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

"QUICK Process"

Your source of the catalase enzyme will be baker's yeast. Baker's yeast, *Saccharomyces cerevisiae*, is a fungus that is used commonly in baking. Yeast is an organism that utilizes the process known as cellular respiration to breakdown glucose (sugar) for energy through a chemical reaction to form water and CO_2. During this process, toxic H_2O_2 slowly builds up as a byproduct and must be broken down in the cell by catalase enzymes.

Key Lab Question: What are some factors that could affect the rate in which catalase enzymes breakdown hydrogen peroxide? (i.e., What factors could **speed up** or **slow down** how quickly catalase works?)

Materials:

- Sharpie
- 6 small Dixie cups and 1 large plastic "waste" cup
- 12 small filter paper disks
- 3 Test tube & test tube rack
- Yeast/enzyme solutions (room temp, salted, chilled, boiled)
- Hydrogen peroxide solution & dropper (pipette)
- Tweezers
- Timer or stopwatch

Procedure:

1. Use a sharpie to label your 6 Dixie cups in the following way: 1)Enzyme @ **room temp**, 2) Enzyme + **SALT**, 3) **Boiled** Enzyme, 4) **Chilled** Enzyme, 5) filter paper disks, 6) LEFTOVER enzyme solutions
2. After your instructor pours each of the 4 enzyme solutions into your appropriate Dixie cup, place 3 filter paper disks into each enzyme solution and leave to soak for ~ 5 minutes

Task 1-Testing Catalase enzymes (Baker's Yeast) **at Room Temperature**

1. Fill each or your 3 test tubes with **2** dropper squirts of hydrogen peroxide solution (about 1 inch).....Adjust the liquid volumes until all 3 test tubes are filled to the SAME height.
2. After your paper disks are well soaked with enzyme, carefully pour the enzyme solution into the LEFTOVER enzyme solutions Dixie cup without losing the 3 paper disks.
3. Using tweezers, place one filter paper disk into the first test tube with the hydrogen peroxide solution. The disk will sink to the bottom. Push it down with the dropper if it sticks to the side of the test tube. Begin timing at the moment the disk touches the **bottom** of the test tube.
4. The disk will eventually float to the surface as it fills with O_2 bubbles produced by the breakdown of H_20_2. Stop timing when the disk reaches the **surface**.
5. Record your time in the appropriate place on **data table 1**.
6. Repeat the above steps two more times and record the times under the columns titled **Trial 2** and **Trial 3**. Once you have finished your three trials, calculate the **average time** and record this in the appropriate column on the data table.
7. Pour the contents of all 3 test tubes into the plastic "waste" cup

Task 2-Measuring Catalase enzymes (Baker's Yeast) **+ Salt**

In this investigation, you will repeat steps #1-7 as you did above, but this time, you will be using a solution of yeast AND salt. What are we testing in this particular investigation?

_____

Record your results in **data table 1** in the row that corresponds to YEAST + SALT.

Task 3-Measuring Catalase enzymes (Baker's Yeast) **that has been BOILED**

In this investigation, you will repeat steps #1-7 as you did above, but this time, you will be using a solution of yeast that has been boiled. What are we testing in this particular investigation?

_____

Task 4-Measuring Catalase enzymes (Baker's Yeast) **that has been CHILLED**

In this investigation, you will repeat steps #1-7 as you did above, but this time, you will be using a solution of yeast that has been chilled. What are we testing in this particular investigation?

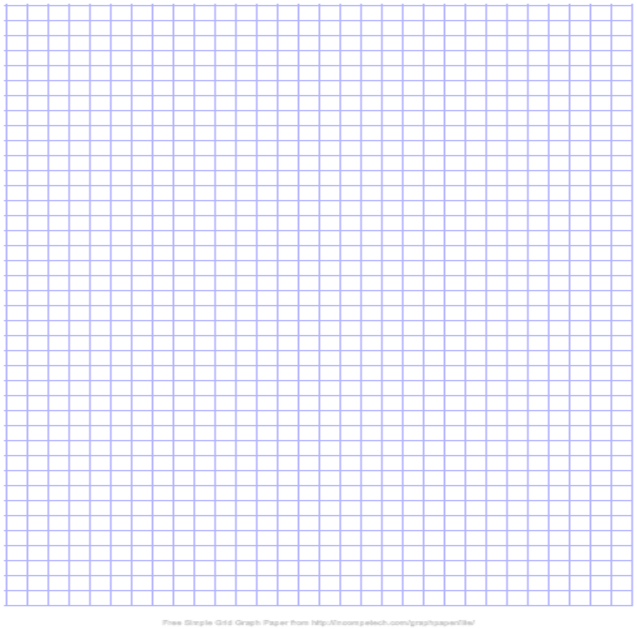
_____

Record your data from your tasks in the data table below, data table 1.

Time (seconds)to surface				
	Trial #1	Trial #2	Trial #3	Average Time
Enzyme @ rm.temp				
Enzyme + SALT				
Enzyme, Boiled				
Enzyme, Chilled				

Graphing Practice:

Create a graph of your results from data table 1 that will COMPARE the **average time** for H_2O_2 to breakdown when catalyzed by a catalase enzyme experiencing 4 different cellular conditions (room temp, salt, boiled, chilled). Graph your data found in data table 1 on the graph provided below. Remember to give your graph an appropriate title, label your axis correctly and completely, and plot your data accordingly. If you need to construct a key, please do so in the space provided to the left of your graph.



Conclusion Questions *Answer the following questions.*

1. Which enzyme and cell conditions took the **least** amount of time for the disk to float? _____

   Using your results and prior knowledge, provide an explanation for your findings:

   _____

   _____

2. Which enzyme and cell conditions took the **longest** amount of time for the disk to float? _____

   What are some possible reasons? _____

   _____

   _____

3. Did any of the enzyme and cell conditions cause the disk **not to float**? _____

   Explain WHY? _____

   _____

Dot Lab Review **Matching**:

4 Choices:	
Hydrogen Peroxide (H_2O_2)	water (H_2O)
Catalase	oxygen (O_2)

catalase
$2\ H_2O_2$ ------> $2\ H_2O\ +\ O_2$

4. Write the name of the **REACTANT** from the DOT lab? _____
5. Write the name of the **PRODUCTS** from the DOT lab? _____
6. Write the name of the substance contained in the "**bubbles**"? _____
7. Write the name of the **liquid** left after the reactions are finished? _____
8. Write the name of the **enzyme** the catalyzes the reaction? _____
9. Write the name of the **substrate** in the reaction? _____
10. Write the name of the substance with the **active site**? _____
11. Write the name of the substance **made from amino acids**? _____
12. Write the name of the substance that can be **denatured**? _____

13. Explain what would happen to your "float time" data and WHY if your 3 test tubes did not have the same exact height of hydrogen peroxide? _____
_____

14. Explain what would happen to your "float time" data if we used old hydrogen peroxide that had been stored on a shelf for 10 years? _____
_____

15. Explain WHY bubbles form when you put hydrogen peroxide on a cut? _____
_____

16. Explain what would happen to your skin cells over time if they did NOT make catalase enzymes? _____
_____

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Teacher Dot lab Demo: **Measuring Catalase enzymes** (Baker's Yeast) **with different pH conditions (ACID or BASE)**

	Time (seconds)to surface				
	pH	Trial #1	Trial #2	Trial #3	Average Time
Enzyme in **ACID**					
Enzyme in water (neutral)					
Enzyme in **Base**					

17. Interpret these results …. Explain how different pH conditions affect the function of catalase?
_____
_____

18. The human stomach is filled with a strong acid, yet the small intestine has basic chemical conditions. Circle where catalase enzymes would function the best (stomach or small intestine) and explain why?
_____
_____