

I've Got That Sinking Feeling

An Investigation of Enzymes

Student Materials

Introduction	2
Lab Protocol.....	4
Pre-Lab Questions.....	7
Data Collection Worksheet.....	8
Post-Lab Questions and Analysis	9

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Sinking Feeling

Introduction

Many chemical reactions require an input of energy to occur. This “get going” energy is called **activation energy**. Special molecules (almost always proteins), called **enzymes**, increase reaction rates by lowering activation energy. A reactant molecule that interacts with an enzyme is called a **substrate**. Enzymes work by binding substrates (reactants) in specific orientations that make them most likely to react, so the reaction rate is much faster than it would otherwise be. Almost all biochemical reactions that occur in living organisms require enzymes to occur quickly enough to sustain life.

Enzymes facilitate all kinds of reactions, reactions that break a substrate down into multiple products, reactions that combine substrates to form a single product, or reactions that structurally alter a substrate in some other way. Generally, enzymes are specific to one reaction, meaning that they act only on a specific substrate or substrates. Their specificity comes from their three-dimensional shape, which includes one or more areas for substrate binding called **active sites**. The fit between the active site and the substrate is often compared to a lock and key—the match must be perfect, or else the reaction will not occur.

When the substrate or substrates bind to the enzyme’s active, an **enzyme-substrate complex** is formed. Once the reaction occurs, the **product(s)** are released and the enzyme is available to bind to more substrate molecules, as shown in **Figure 1**.

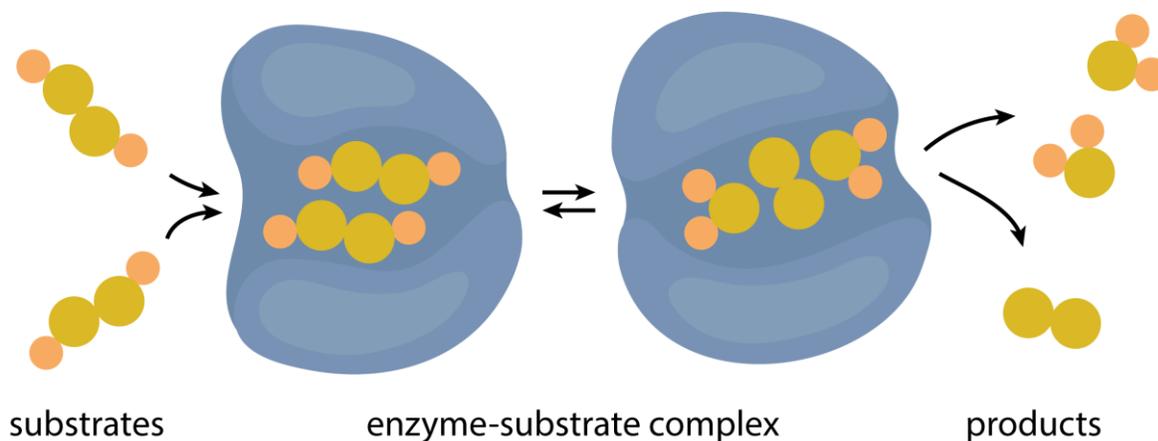


Figure 1. Mechanism of enzyme activity. One or more substrates bind at the enzyme’s active site forming an enzyme-substrate complex. The enzyme induces a chemical change in the substrate(s), and then releases the product(s).

Reactions that involve enzymes are called enzyme-catalyzed reactions. This is because enzymes are catalysts, meaning that they are involved in a chemical reaction, but are not consumed by it. Once the reaction is complete, the enzyme is exactly as it was before the reaction started, meaning that a single enzyme can be used over again.

One example of an enzyme-catalyzed reaction is the breakdown of hydrogen peroxide to water and oxygen, represented by the equation below.



Hydrogen peroxide is generated as a waste product in the cells of nearly all living organisms. Although it is a byproduct of many normal chemical reactions, it is toxic to cells in high doses. Hydrogen peroxide

accumulation can result in cell death, so it must be broken down quickly and efficiently to prevent build-up. The enzyme that catalyzes the breakdown of hydrogen peroxide is called **catalase**.

Interestingly, cellular catalase levels decrease as a natural part of the aging process. One visible consequence of decreased catalase (and therefore increased cellular hydrogen peroxide) is grey hair. Hydrogen peroxide inhibits the synthesis of color pigments and bleaches hair from within the follicle. Without sufficient catalase to break it down, hydrogen peroxide builds up in hair cells and leaves its mark by diminishing color pigments.

Cells have hundreds of different enzymes, each of which catalyzes a particular chemical reaction. How fast these enzyme-catalyzed reactions occur depend on many factors, including temperature, pH, and the concentrations of the substrates and the enzyme.

In this lab, you will examine the effect of catalase concentration on the rate of hydrogen peroxide breakdown. You will prepare filter paper disks soaked in different concentrations of catalase and incubate the disks in a hydrogen peroxide solution. As the catalase breaks down the hydrogen peroxide, oxygen bubbles will build up on the disk and cause the disk to float to the top of the solution. The speed with which the catalase-soaked disk floats to the surface of hydrogen peroxide solution is an indicator of how quickly the chemical reaction is proceeding.

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Lab Protocol

Materials: Check your workstations to make sure all supplies are present before beginning the lab.

Student Workstation:		Common Workstation:
150 mL	hydrogen peroxide solution (0.3%)	Extra hydrogen peroxide (0.3%) Extra dH ₂ O
5 mL	catalase stock solution (100 %)	
5 mL	distilled water	
1	p20 micropipette with tips	
1	p200 micropipette with tips	
1	p1000 micropipette with tips	
1	24-well plate	
2	20 mL clear vials	
1	single hole paper punch	
1	forceps	
1 sheet	filter paper	
1	stop watch or timer	
1	permanent marker	
3+	paper towels	

Procedure:

1. Make 20 disks by punching holes in the filter paper with the paper punch.
2. Complete **Table 1** below to determine how much of the 0.4 mg/mL catalase stock solution and how much distilled water you will need to make each of the required catalase dilutions. Use the following formula:

$$(\text{concentration 1})(\text{volume 1}) = (\text{concentration 2})(\text{volume 2}) \text{ OR}$$

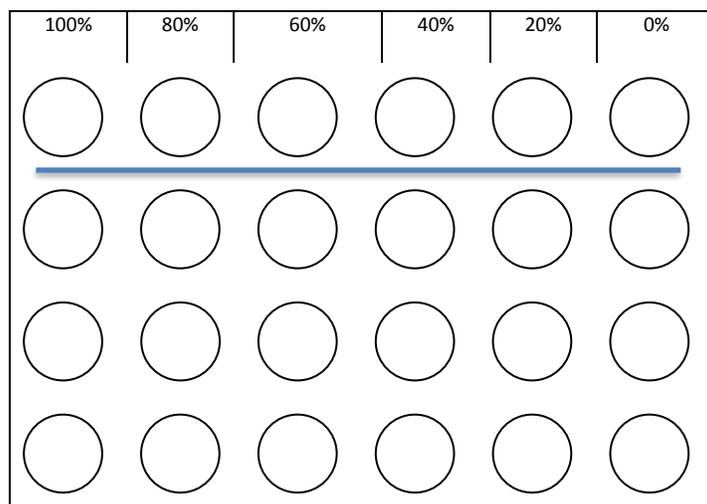
$$C_1V_1 = C_2V_2$$

Example: $(80\%)(500 \mu\text{L}) = (80\%)(x \mu\text{L})$; Solve for $x = 400 \mu\text{L}$; Add 400 μL of the 100% solution + 100 μL water to make 500 μL of 80% solution.

Table 1. Catalase Dilutions.

Final quantity needed (μL)	Final catalase solution concentration	Microliters (μL) of 0.4 mg/mL catalase stock solution	Microliters (μL) of distilled water
500	100%	500	0
500	80%	400	100
500	60%		
500	40%		
500	20%		
500	0%		

3. Use a permanent marker to label the wells in the first row of the multi-well plate with the final catalase solution concentrations listed in **Table 1**. Your well plate should look like the one in below.



4. Using a p1000 micropipette, put 500 μL of distilled water into the plate well labeled 0%
About how full is the well? _____
5. Use **Table 1** as a guide and use a p200 or p1000 micropipette to add the appropriate amount of distilled water to the other dilution wells.
6. Using a p1000 micropipette with a **clean pipette tip**, put 500 μL of 100% catalase solution into the plate well labeled 100%.
7. Use **Table 1** as a guide and use a p200 or p1000 micropipette to add the appropriate amount of catalase to the other dilution wells.
8. Fill each of two clear vials with 20 mL (milliliters and not microliters) of hydrogen peroxide solution. Label one of the vials 0%.
9. Using the forceps, pick up one filter paper disk. Use a p20 micropipette to transfer 10 μL of distilled water onto the disk.
10. Record the time in Data Collection Table (on the Data Collection Table Worksheet), in the space for "Starting Time," then, use the forceps to place the water-soaked disk onto the surface of the hydrogen peroxide solution in the vial labeled 0%. The disk should sink to the bottom of the vial.
11. Set the vial to the side. **▲ Caution: Do not to disrupt the disk.**

12. Using the forceps, pick up a new filter paper disk and hold it while you add 10 μL of the 100% catalase solution from the well onto the disk. **▲ Caution: Use a clean tip.**
13. Using the forceps, place the catalase-soaked disk onto the surface of the hydrogen peroxide solution in the second of the two vials you prepared.
14. Using the stopwatch, measure the time it takes for the disk to sink to its lowest point in the vial and then rise back to the surface of the solution. Record your data in the Data Collection Table.

▲ Important: Coordinate with your lab partner to begin timing as soon as the disk touches the surface of the hydrogen peroxide solution.

15. Once the disk rises back to the surface of the hydrogen peroxide solution and the trial is complete, use the forceps to remove the disk from the vial. The used paper disk can be disposed of in the trash.
16. Repeat steps 12–15 two more times for the 100% catalase dilution, using a new filter paper disk each time.

▲ Caution: To prevent contamination, be sure to wipe the forceps clean with the paper towel *each time* you prepare a disk and change pipette tips after *each trial* of the experiment!

17. After you have completed all three trials at a given catalase concentration, dispose of the hydrogen peroxide solution by pouring it down the drain. Rinse your vial once with distilled water and refill with 20 mL of fresh hydrogen peroxide.
18. Repeat steps 10–15 for the remaining dilutions: 80%, 60%, 40%, and 20%, performing a set of 3 trials for each concentration.

▲ Caution: Remember to use fresh hydrogen peroxide for each set of 3 trials. Use a clean tip for each trial.

19. Once you have completed all trials, record the time in the Data Collection Table under “Ending Time.” Examine the water-soaked disk sample and record your observations here.

Did the disk move from the starting point? _____

20. Calculate the average of the three trials for each catalase concentration and record the average (in seconds) in the Data Collection Table.

Sinking Feeling

Pre-Lab Questions

Directions: After reading through the introduction and protocol for the Sinking Feeling lab, answer the questions below.

1. Why are enzymes important to all living things?
2. Explain how an enzyme and substrate are like a lock and key.
3. Why can just a small amount of enzyme have a dramatic effect on a reaction's rate?
4. Describe the role that catalase has in living cells.
5. Identify the dependent and independent variables for this experiment. What is the control?
6. Propose a hypothesis about the effect catalase concentration will have on the rate at which hydrogen peroxide is broken down.

Sinking Feeling

Data Collection Worksheet

Name: _____

Other group members: _____

Class/period: _____

Date: _____

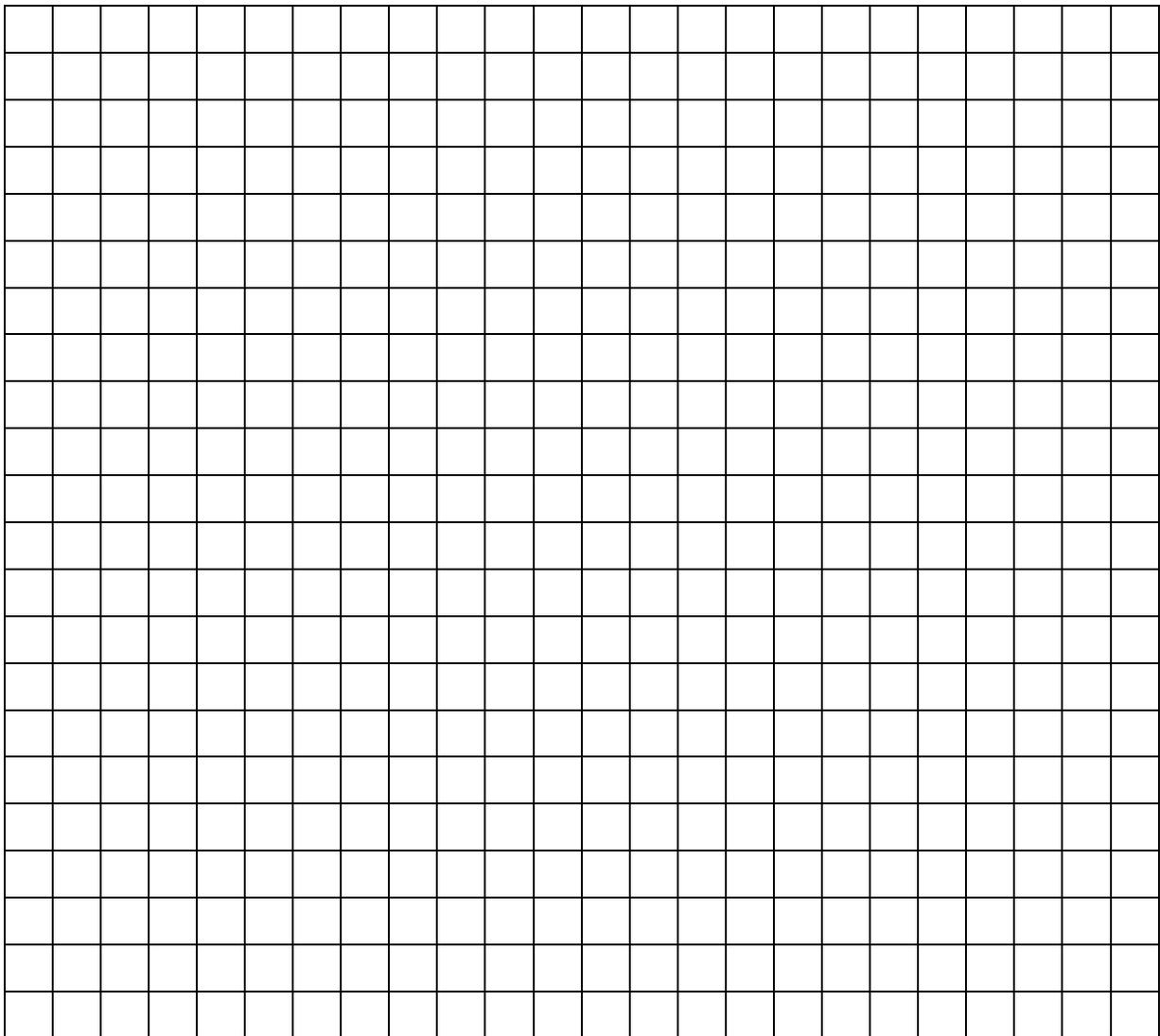
Catalase	Starting Time	Ending Time	Total Time (min)	
0% (water)				
Time (s) for Disk to Sink and Rise to Surface				
Catalase concentration	Trial 1	Trial 2	Trial 3	Average Time
100%				
80%				
60%				
40%				
20%				

Sinking Feeling

Post-Lab Questions and Analysis

Directions: After completing the Sinking Feeling lab, answer the questions below.

1. Why did you measure the time it takes the disks to sink and then rise to determine the reaction rate?
2. Use your data to construct a bar graph that shows the reaction rate versus the enzyme concentration. Be sure to label your x-and y-axes clearly, use an appropriate scale and units, and to give your graph a title.

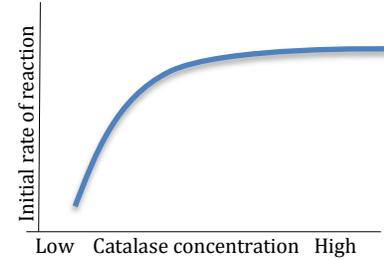
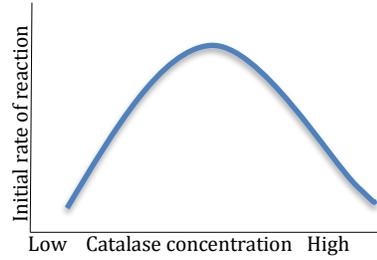
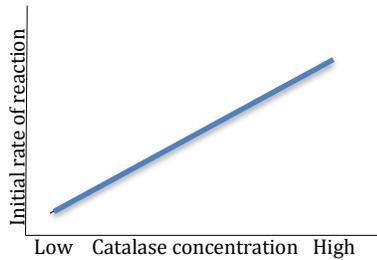


3. Based on the graph you generated, what can you conclude about the effect of enzyme concentration on reaction rate? Does the data support, or refute your hypothesis?

4. Explain why catalase concentration affected the chemical reaction rate.

5. If you were to repeat the experiment with higher and higher catalase concentrations, which of the following graphs shows the generalized trend that you would predict?

Explain your answer.



6. Describe **three** potential sources of error in this experiment. Be specific.

7. Was this experiment a valid way to measure the effect of enzyme concentration on chemical reaction rate? Explain your answer.

8. Predict what would happen in this experiment if you had kept catalase concentration constant, but instead varied the hydrogen peroxide concentration. Explain your answer.