

# Catalytic Activity of Enzymes

## Introduction

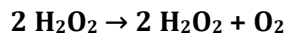
Enzymes are biological molecules that catalyze (speed up) chemical reactions. They are the “Builders and Do-ers” in the cell; without them, life could not occur. Every cell makes hundreds of different enzymes to carry out the reactions necessary for life. Fortunately for the cell, enzymes are not used up when they catalyze a reaction, but can be used over and over.

The molecule (or molecules) on which an enzyme acts is called its **substrate**. Enzymes are said to be very “specific,” meaning that they recognize only one substrate (or a few closely related substrates) and convert it into a specific product. Each enzyme is specific because it is folded into a particular three-dimensional shape. Within the folds of each enzyme is the **active site**, the place where the substrate fits and where the chemical reaction takes place.

Enzymes work very quickly, often catalyzing thousands of reactions per minute. The rate at which an enzyme works is influenced by many factors including temperature and pH. Enzymes have a temperature and pH at which they work best, and if an enzyme is exposed to extremes of heat or pH it won't work at all! The interactions that hold the protein in its particular shape become disrupted under these extreme conditions, and the 3- dimensional structure unfolds. In this case, the enzyme is said to be **denatured**. Other important factors that influence enzyme activity are the concentration of substrate and the concentration of enzyme. Up to a point, the more substrate that is present, the faster the reaction. However, when the substrate concentration is so high that an enzyme is working as fast as it can, further increases of substrate concentration will have no effect on the rate of product formation.

## Background

The enzyme that you will study in this experiment is **catalase**. Its job is to break down its substrate hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), which is a naturally occurring poison. Without catalase,  $\text{H}_2\text{O}_2$  could kill the cell. The reaction catalyzed by catalase is:



The products remaining after catalase does its job are oxygen gas and water; two very non-poisonous molecules.

In the home and hospital, hydrogen peroxide is used as an antiseptic to clean out wounds. Have you ever noticed that when hydrogen peroxide is swabbed on a cut it bubbles? This is because enzymes in the cut from your body and from infecting bacteria catalyze the rapid degradation of hydrogen peroxide into water and oxygen. The bubbles are oxygen.

Catalases are very common, they are found in almost all cells that grow in oxygen, including potato tubers. In this experiment, a blender is used to grind up a potato in water to release the catalase from the potato cells. The ground-up potato is filtered through cheesecloth to separate potato skin and cell debris from the liquid which contains most of the cell's enzymes, including catalase. To actually measure the catalase activity, small disks are dipped into the potato cell extract. When this enzyme-containing disk is placed in a solution of hydrogen peroxide, the enzyme begins to work. As the catalysis occurs, oxygen is produced, and bubbles of the gas become trapped in the fibers of the disk. When there are enough  $\text{O}_2$  bubbles, they lift the filter to the surface. The speed with which the  $\text{O}_2$  is produced depends both upon how much enzyme is present and on the concentration of the hydrogen peroxide. The more enzyme, the faster the product ( $\text{O}_2$ ) is made. Similarly, the

higher the concentration of the substrate, hydrogen peroxide, the faster the product is made. You can see what happens when you vary either the concentration of enzyme or the concentration of the hydrogen peroxide.

To do this experiment, half of the teams of students will carry out one version of the experiment using low, medium, and high enzyme concentration and a constant level of substrate, 1.0%  $\text{H}_2\text{O}_2$ . Then, the other half of teams will do another version of the experiment using low, medium, and high  $\text{H}_2\text{O}_2$  concentration with cell extract diluted to 60% of its original concentration. At the end of the lab, experimental results will be pooled and the class as a whole will generate data showing the relationship between catalase activity and both enzyme and substrate concentrations.

A third experiment dealing with the effect of pH is provided. This portion could be performed easily by one or two teams of students. It also lends itself nicely to a teacher demonstration for the entire class.

For an experiment to be meaningful, there must be controls. Three controls important to this lab will be demonstrated by your teacher:

**Control #1:** A paper disc that **HAS NOT** had potato extract added to it is dipped in  $\text{H}_2\text{O}_2$ .

**Control #2:** A paper disc that **HAS** been dipped in potato extract is placed in a beaker of water.

**Control #3:** A paper disc that **HAS FIRST** been dipped in boiled potato extract **AND THEN** placed into a beaker of  $\text{H}_2\text{O}_2$ .

Observe and record on what occurs as each control experiment is demonstrated. Why is each control important?

What does each control experiment show you?

### **Materials (work as a team)**

- potato extract (prepared by your teacher)
- 400 ml flask with distilled water
- 1 - 250 ml beaker for potato extract
- 200 ml 1 %  $\text{H}_2\text{O}_2$  solution for the first part of the experiment
- 3%  $\text{H}_2\text{O}_2$  solution to dilute for second part of the experiment
- 100 ml graduated cylinder
- forceps
- paper towels
- 60% catalase
- 4 small beakers or cups
- 10 filter paper disks
- stop watch
- calculator

## Part A. Various Catalase Concentrations

1. Obtain the potato extract from your teacher:
  - This extract will be designated as “100%” catalase.
  - Each team should have a 250 ml beaker containing 200 ml of 100% catalase.
2. Together with your partners, prepare your enzyme concentrations in the beakers:
  - Label the beakers: 10%, 20%, 40%, 60%, 80%, and 100%.
  - Make the appropriate dilutions. For example, if you are doing test #1 (10%), measure 4.0 ml of the potato extract using the graduated cylinder and pour into the beaker.
  - Rinse the graduated cylinder, then add 32.0 ml of distilled H<sub>2</sub>O and mix well with the stirring rod.
  - Make the rest of the enzyme solutions using the chart below as a guide.

Test	Extract Concentration	Volume of Enzyme	Volume of Water
#1	10%	4 ml	36 ml
#2	20%	8 ml	32 ml
#3	40%	16 ml	24 ml
#4	60%	24 ml	16 ml
#5	80%	32 ml	8 ml
#6	100%	40 ml	0 ml

3. You are now ready to begin measuring the effects of enzyme concentration on enzyme activity:
  - Obtain the flask of 1% hydrogen peroxide. (This is diluted down from the original 3%.)
  - Pour 30 ml of the 1% H<sub>2</sub>O<sub>2</sub> solution into a clean beaker, labeled “reaction beaker.”
  - Pick up a paper disk with a clean forceps. Using the forceps, dunk the disk in your enzyme extract for FIVE (5) seconds, until the disk is uniformly moistened but not beaded with shiny drops of liquid.
  - Drain it on a piece of paper towel for 5 seconds to remove excess enzyme from the disk.
4. The reaction is now ready to be started and timed:
  - Using forceps, place the filter disk (containing the enzymes) onto the **bottom** of the “reaction beaker” containing 1% hydrogen peroxide. NOTE: Your disk may not sink all the way.
  - One person should watch the clock/stopwatch, another watch the rising disk. Stop timing as soon as disks have completely ‘lifted off’ the bottom of the reaction beaker.
  - Watch the filter disk. You should see tiny bubbles of oxygen being released as the hydrogen peroxide is broken into water and oxygen by the catalase.
  - Record the time in seconds for each trial on the chart.
  - Remove the disk and discard it.
5. Obtain another disk and repeat steps 3-4 exactly as done above.
6. Repeat the experiment a third time exactly as above: now you have “triplicate” measurements of the rate of oxygen production in 1% H<sub>2</sub>O<sub>2</sub> at each particular enzyme concentration. Average these 3 values and record in the chart. Repeat this procedure for all the concentrations of enzyme.

**Table 1. Enzyme Concentration**

Test Number	Trial 1	Trial 2	Trial 3	Team Average	Class Average
1. 10%					
2. 20%					
3. 40%					
4. 60%					
5. 80%					
6. 100%					

**Part B. Various Hydrogen Peroxide Concentrations**

- Label the clean beakers with the percent hydrogen peroxide that will be used in this part of the experiment: 2%, 1.5%, 1.0%, 0.8%, 0.6% and 0.3%. Together with your partner, prepare your particular substrate concentrations in each of the labeled beakers. For example, for test #1, measure 20.0 ml of the concentrated H<sub>2</sub>O<sub>2</sub> using the graduated cylinder and pour into the beaker. Add 10 ml of distilled water to the graduated cylinder and pour into the hydrogen peroxide. Stir well with the stirring rod. Rinse the graduated cylinder. See chart for proportions of hydrogen peroxide and water to mix for each dilution. Place all of the beakers on the table in front of you in order from lowest to highest concentration of hydrogen peroxide.

Test #	Substrate Concentration	Volume of H <sub>2</sub> O <sub>2</sub>	Volume of Water
1	2.0%	20 ml	10 ml
2	1.5%	15 ml	15 ml
3	1.0%	10 ml	20 ml
4	0.8%	8 ml	22 ml
5	0.6 %	6 ml	24 ml
6	0.3 %	3 ml	27 ml

- Next, obtain your 60% catalase solution. (This was diluted from the original 100%.)
- Using forceps, dip a disk in the 60% potato extract for 5 seconds, let it drain on a paper towel for 5 seconds. Then, using forceps, place the filter (containing enzymes) on the bottom of the "reaction" beaker (2.0% H<sub>2</sub>O<sub>2</sub>).
- Time how long it takes the disk to rise from the bottom of the beaker to the top of the liquid. Be sure that the disk is placed at the bottom of the hydrogen peroxide before you start to time the experiment.
- Record the time in seconds in the appropriate space on the chart that follows.
- Obtain another disc and repeat steps 3a - 3c exactly as before.
- Repeat the experiment a third time. Average these three values and record on the chart.
- Repeat this procedure for all of the concentrations of H<sub>2</sub>O<sub>2</sub>.

**Table 2. Substrate Concentration**

Test Number	Trial 1	Trial 2	Trial 3	Team Average	Class Average
1. 2%					
2. 1.5%					
3. 1%					
4. 0.8%					
5. 0.6%					
6. 0.3%					

**After the Experiment**

Pool your results with those of the rest of the class, record below and fill in the class average portion of the table for your experiment and also for the other experiment.

**Table 3. Enzyme Concentration (mean of 3 trials for each test)**

test	team 1	team 2	team 3	team 4	team 5	team 6	Average
1. 10%							
2. 20%							
3. 40%							
4. 60%							
5. 80%							
6. 100 %							

**Table 4. Substrate Concentration (mean of 3 trials for each test)**

test	team 1	team 2	team 3	team 4	team 5	team 6	Average
1. 2.0%							
2. 1.5%							
3. 1.0%							
4. 0.8%							
5. 0.6%							
6. 0.3%							

2. Graph #1: Plot the results from Table 3 onto graph paper. Compare both your team and the class averages. The  $x$ -axis is designated enzyme concentration, starting at point 0, while the  $y$ -axis is **1/time**, so do the math on this before you graph your data.

3. Graph #2: Plot the results from Table 4 onto graph paper. Compare both your team and the class averages. The  $x$ -axis is designated substrate concentration, starting at point 0, while the  $y$ -axis is again **1/time**, so do the math first.

### Part C. pH Influence on Enzyme Activity

#### Materials

- six test tubes
- graph paper
- metric ruler
- test tube rack
- pH solutions of 2-4-6-7-8-10
- 3% hydrogen peroxide
- 60% catalase solution

#### Procedure

1. Obtain six test tubes and a test tube rack. Label them pH 2, pH 4, pH 6, pH 7, pH 8, pH 10.
2. Using a dropper pipette, measure 4 ml of each of the pH solutions provided and transfer into the corresponding test tube. To avoid contamination use a different dropper for each solution.
3. To each of the test tubes, add 2 ml of 60% catalase solution. Swirl the test tube to help mix the pH solution with the catalase. Allow to sit for 5 minutes.
4. Measure 1 ml of 3% hydrogen peroxide into each of the test tubes. Allow to react for 5 minutes. Foam should form on the top of the solutions.
5. Measure the distance from the bottom of the test tube to the top of the foam in millimeters and record below.

**Table 5. pH Influence**

pH	2	4	6	7	8	10
height (mm)						

6. Graph #4: Plot your results, using the x-axis for “pH” and the y-axis for “height of foam (mm)”.

#### Questions

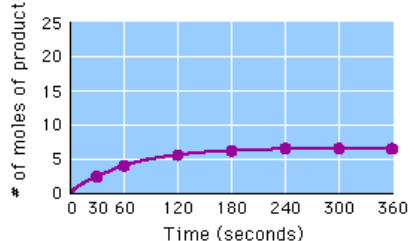
1. What is the function of a control? Explain the purpose for control #1, control #2, and control #3.
2. Describe the effect of enzyme concentration on enzyme activity. Calculate the rate of the reaction (refer to Graph #1).
3. Describe the effect of substrate concentration on enzyme activity. Refer to Graph #2.
4. Describe the effect of pH on enzyme activity. Refer to Graph #3.
5. After an enzyme is mixed with its substrate, the amount of product formed is determined at 10-second intervals for 1 minute. Graph #4: Plot the data from this experiment:

Time (sec)	0	10	20	30	40	50	60
Product formed (mg)	0.00	0.25	0.50	0.70	0.80	0.85	0.85

- a. What is the initial rate of this enzymatic reaction? Show your calculation.
- b. What is the rate after 50 seconds? Calculate the rate of the reaction (use data from Graph #4). Why is it different from the initial rate?

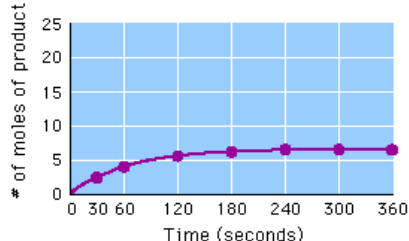
## Sample Lab Exam Questions

1. During what time interval is the enzyme working at its maximum velocity?



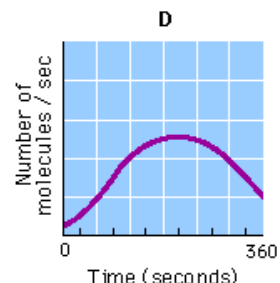
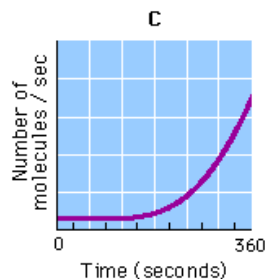
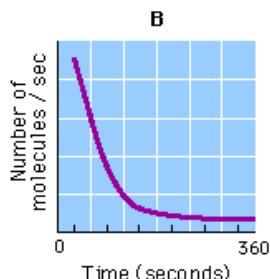
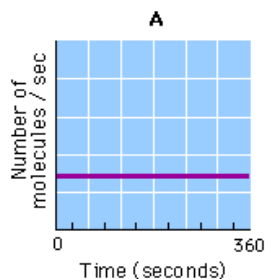
- 0–30 seconds
- 60–120 seconds
- 120–180 seconds
- Over the entire time course

2. In order to keep the rate constant over the entire time course, which of the following should be done?

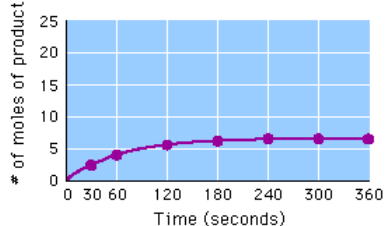


- Add more enzyme.
- Gradually increase the temperature after 60 seconds.
- Add more substrate.
- Add  $\text{H}_2\text{SO}_4$  after 60 seconds.

3. Which of the following graphs represents the rate of the reaction shown above in question #2? Notice that in the graphs below, the y-axis is number of molecules/sec.

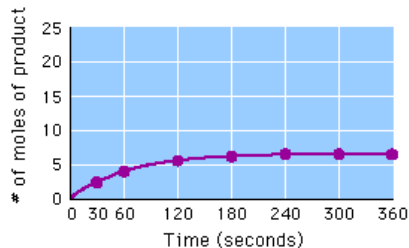


4. What is the role of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) in this experiment?



- It is the substrate on which catalase acts.
- It binds with the remaining hydrogen peroxide during titration.
- It accelerates the reaction between enzyme and substrate.
- It blocks the active site of the enzyme.
- It denatures the enzyme by altering the active site.

5. A student was performing a titration for this laboratory, and accidentally exceeded the endpoint. What would be the best step to obtain good data for this point?



- Estimate the amount of  $\text{KMnO}_4$  that was in excess, and subtract this from the result.
- Repeat the titration using the reserved remaining sample
- Obtain data for this point from another lab group.
- Prepare a graph of the data without this point, and then read the estimated value from the graph.

## AP Lab: Catalytic Activity of Enzymes write-up

(Written portion must be typed, using 12 pt Times New Roman font, 1-inch margins)

### Question:

What is the objective and/or problem being tested?

### Hypothesis:

Briefly describe the experimental design and your prediction of results (Use “If ..., then ...” statement.) Formulate a hypothesis for each of the experiments: Part A, Part B, and Part C.

### Procedure:

Write: *See lab handout*

### Data:

- Table 1, Table 2, Table 3, Table 4, Table 5
- Graph 1, Graph 2, Graph 3, Graph #4
- Calculations of rate

### Analysis:

Answer questions #1-5 and sample exam questions #1-5.

### Conclusion:

Paragraph 1: Overall statement of findings (data/observations) you made. Give actual numbers and figures from the experiments.

Paragraph 2: (Hypothesis & Explanation) Explain how the evidence supports your hypothesis in both experiments (OR give possible explanation for why the evidence does NOT support your hypothesis). Name any variables in the experiment that can influence the results (control, constant, independent, and dependent.).

Paragraph 3: (Errors & Future) Describe any sources of errors or other factors that could have led to unexpected results or ambiguous data collection, and how they could be avoided. Briefly describe what further experiments should be done and why.