



## Catalytic Activity of Enzymes

### Introduction

Enzymes are biological molecules that catalyze (speed up) chemical reactions. You could call enzymes 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.

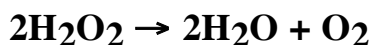
The DNA in each cell encodes all the information needed to make its many different enzymes. Enzymes are relatively large molecules of protein. They are produced whenever the cell “senses” a need for that particular enzyme; that is, whenever a job needs to be done in the cell which only that enzyme can do it.

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. You could say that each enzyme can do only one type of job. 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 for this lab

The enzyme that you will study in this experiment is called “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, five 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 six 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 page 8 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)**

Your team will need the following supplies:

- potato extract (prepared by your teacher)
- 1000 ml flask with distilled/deionized/dechlorinated 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
- 8 - 100 ml beakers
- forceps
- 40 filter paper disks
- paper towels
- stop watch if available
- 60% catalase
- calculator

## Experimental Procedure for the Teams Working with the Various Catalase Concentrations

### 1. Making the potato extract:

- Watch your teacher prepare the potato extract as follows:
- Cut clean potatoes into chunks (allow one potato per team of students)
- Place the potato chunks in the blender and add 200 ml of buffer per potato.
- Puree in the blender.
- Pour potato puree through four layers of cheesecloth placed in funnel. Collect as much fluid as possible. This fluid contains the enzyme **catalase**, among many other things that were stored inside the cells of the potato.
- Add enough distilled water to bring the final volume to 200 ml per potato. Swirl the flask to mix the solution. This will be arbitrarily designated as “100%” catalase extract. (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 with tape and pen: 20%, 40%, 60%, 80%, and 100%.
- Make the appropriate dilutions. For example, if you are doing test #1(20%), measure 8.0 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 stir 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	20%	8 ml	32 ml
#2	40%	16 ml	24 ml
#3	60%	24 ml	16 ml
#4	80%	32 ml	8 ml
#5	100%	40 ml	0 ml

- Obtain the flask of 1% hydrogen peroxide if it is not at your table. This is the substrate for this part of the lab.

3. Now you are ready to begin measuring the effects of enzyme concentration on enzyme activity.
  - Pour 30 ml of the 1%  $\text{H}_2\text{O}_2$  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.
  - 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. Be precise in your timing and recording.
  - 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%  $\text{H}_2\text{O}_2$  at each particular enzyme concentration. Average these 3 values and record in the chart. Repeat this procedure for all the concentrations of enzyme.

### Data Table / Enzyme Concentration

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

- Clean up all materials!

## After the Experiment

### Controls

A. What is the function of a control?

For control #1, a filter paper saturated with water rather than potato extract was placed in a beaker of 1%  $\text{H}_2\text{O}_2$ . How long does it take for the filter to lift off? \_\_\_\_\_  
Explain the significance of the result:

For control #2, a piece of filter paper was saturated with potato extract and then placed in distilled water. How long did it take for the filter to lift off? \_\_\_\_\_  
Explain the significance of the results:

For control #3, 100% catalase was boiled. A filter paper was then saturated with this extract. The disk containing the extract was then placed in a beaker of 1%  $\text{H}_2\text{O}_2$ . How long did it take for the filter to lift off? \_\_\_\_\_ Explain significance of the results:

B1. 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.

### Data Table for Enzyme Concentration

(mean for each test)

test	team 1	team 2	team 3	team 4	team 5	team 6	team 7	team 8
1: 20%								
2: 40%								
3: 60%								
4: 80%								
5: 100%								

### Data Table for Substrate Concentration

(mean for each test)

test	team 1	team 2	team 3	team 4	team 5	team 6	team 7	team 8
1: 2.0%								
2: 1.5%								
3: 1.0%								
4: 0.8%								
5: 0.6%								
6: 0.3%								

- Plot both the team and the class averages on graph paper. The first graphs should be “concentration of enzyme vs. time of reaction.” The  $x$ -axis is designated concentration, starting at point 0. The  $y$ -axis is  $1/t$ , so you have to do the math on this before you graph your data. The second graph should be “concentration of substrate vs. time of reaction.” The  $x$ -axis is designated concentration, starting at point 0. The  $y$ -axis is again  $1/t$ , so do the math first.



3. Discuss **three** factors that influence the rate of enzyme action:

4. Why did you do the experiment in triplicate?